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ORIGINAL ARTICLES

THE INTERNAL SECRETION OF THE PANCREAS*

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THE hypothesis underlying this series of experiments was first formulated by one of us in November, 1920,† while reading an article dealing with the relation of the isles of Langerhans to diabetes.[1] From the passage in this article, which gives a résumé of degenerative changes in the acini of the pancreas following ligation of the ducts, the idea presented itself that since the acinous, but not the islet tissue, degenerates after this operation, advantage might be taken of this fact to prepare an active extract of islet tissue. The subsidiary hypothesis was that trypsinogen or its derivatives was antagonistic to the internal secretion of the gland. The failures of other investigators in this much-worked field were thus accounted for.

The feasibility of the hypothesis having been recognized by Professor J. J. R. Macleod, work was begun, under his direction, in May, 1921, in the Physiological Laboratory of the University of Toronto.

In this paper no attempt is made to give a complete review of the literature. A short résumé, however, of some of the outstanding articles which tend to attribute to the isles of Langerhans the control of carbohydrate metabolism, is submitted.

In 1889 Mering and Minkowski[2] found that total pancreatectomy in dogs resulted in severe and fatal diabetes. Following this, many different observers experimented with animals of various species and found in all types examined, a glycosuria and fatal cachexia after this operation. The fact was thus established that the pancreas was responsible for this form of diabetes. In 1884, Arnozan and Vaillard[3] had ligated the pancreatic ducts in rabbits and found that within twenty-four hours the ducts become dilated; the epithelial cells begin to desquamate; and that there are protoplasmic changes in the acinous cells. On the seventh day there is a beginning of

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round-celled infiltration. On the fourteenth day the parenchyma was mostly replaced by fibrous tissue. Sscobolew[4] in 1902 noted in addition to the above, that there was a gradual atrophy and sclerosis of the pancreas with no glucosuria. However, in the later stages, from thirty to one hundred and twenty days after ligation of the ducts, he found involvement of the islets and accompanying glucosuria.

Lewaschew[5] believed that the islets were modified acinous cells. Laguesse,[6] an anatomist, first suggested that the islets might be the organ of pancreatic internal secretion. He showed that there were comparatively more islets in the fetus and the newborn than in the adult animal. Opie[7] and Sscobolew[8] independently furnished the first clinical foundation for the belief that the islets were involved in pancreatic diabetes.

W. G. MacCallum,[9] in 1909, ligated the ducts draining the tail third of the pancreas. After seven months he excised the remaining two-thirds. This was followed by a mild glucosuria. Three weeks later he removed the degenerated tail third. This second operation resulted in an extreme and fatal glucosuria. Kirkbridge,[10] in 1912, repeated and corroborated MacCallum's findings and, by the use of Lane's[11] method of staining, proved that the atrophic tissue contained healthy islets.

Kamimura[12] in 1917, working on rabbits, traced the degenerative changes in the parenchymatous tissue of the pancreas after ligation of the ducts, and found that the islets remained normal and that the animal did not develop glucosuria as long as the islets were left intact.

The first attempt to utilize the pancreas in defects of carbohydrate metabolism was made by Minkowski.[13] This worker tried the effect of pancreatic feeding, with no beneficial results. Up to the present time only useless or even harmful effects have been obtained from repeated attempts to use this method.

Knowlton and Starling,[14] in 1912, published experiments which showed a marked decrease in the power of using sugar of a diabetic heart perfused outside the body, as compared with a normal heart under similar conditions. Macleod and Pearce,[15] using eviscerated animals were unable to confirm the above results. Patterson and Starling[16] subsequently pointed out that a serious error was involved in the early experiments due to (1) excess glycogen present in diabetic hearts, and (2) to the irregular disappearance of glucose from the lungs.

Murlin[17] prepared an alkaline extract of pancreatic tissue and after injection of this solution, secured a reduction in sugar excreted in a diabetic animal. Kleiner[18] has pointed out that the reduction secured by Murlin might be due to the alkali *per se*. Kleiner himself has shown that "unfiltered-water extracts of fresh pancreas diluted with .90 per cent NaCl when administered slowly usually resulted in a marked decrease in blood sugar." There was no compensating increase in urine sugar, but rather a decrease, which Kleiner suggests may be partly due to a temporary toxic renal effect. Hemoglobin estimations made during the experiment showed that the reduction in blood sugar was not a dilution phenomenon. Paulesco[19] has recently demonstrated

the reducing effect of whole gland extract upon the amounts of sugar, urea and acetone bodies in the blood and urine of diabetic animals. He states that injections into peripheral veins produce no effect and his experiments show that second injections do not produce such marked effect as the first.

From the work of the above-mentioned observers we may conclude: (1) that the secretion produced by the acinous cells of the pancreas are in no way connected with carbohydrate utilization; (2) that all injections of whole-gland extract have been futile as a therapeutic measure in defects of carbohydrate utilization; (3) that the islands of Langerhans are essential in the control of carbohydrate metabolism. According to Macleod there are two possible mechanisms by which the islets might accomplish this control: (1) the blood might be modified while passing through the islet tissue, i.e., the islands might be detoxicating stations and (2) the islets might produce an internal secretion.

We submit the following experiments which we believe give convincing evidence that it is this latter mechanism which is in operation.

In the ten-week interval which we considered necessary for complete degeneration of the acinous tissue, we secured records of dogs depancreatized by the Hédon method.[20]

METHODS

The first chart is a record of an animal depancreatized by the Hédon method. The details of this operation are given in Hédon's article.[20] The remaining records are of animals (females) completely depancreatized at the initial operation. The procedure is as follows: under general anesthesia an upper right rectus incision is made through the abdominal wall. The duodenum is delivered through the abdominal wound, and the pancreas traced to the tail portion. The mesentery beyond is cut between clamp and ligature. Vessels from spleen are then isolated, ligated and divided. Little dissection is then required until the duodenum is reached. The superior pancreaticoduodenal vessels are located and great care is exercised to avoid damaging them. The pancreas is stripped from the duodenum by dry dissection. The vessels to the uncinate process are ligated and divided, and the process freed from its mesenteric attachments. The larger duct of the pancreas is then ligated close to its entry into the duodenum and the pancreas is removed. Special care must be exercised to preserve the splenic vessels. The superior pancreaticoduodenal vessels must be left intact. Failing this, duodenal ulcer is a frequent development. If this procedure is carried out the whole gland with the exception of the portion in contact with the duodenum is covered with mesentery. The abdominal wound is closed layer by layer with catgut. A collodion dressing is used. The urethral orifice is exposed by a midline incision of the perineum and the edges of the wound drawn together to facilitate healing.

We have found that animals between eight and sixteen months old are the most suitable for this operation. At this age the pancreas is not so firmly fixed as it becomes later.

We first ligated, under general anesthesia, the pancreatic ducts in a

number of dogs. (Blood sugar estimations on these animals were recorded from time to time. We have no record of a hyperglycemia).

The extract was prepared as follows: The dog was given a lethal dose of chloroform. The degenerated pancreas was swiftly removed and sliced into a chilled mortar containing Ringer's solution. The mortar was placed in freezing mixture and the contents partially frozen. The half frozen gland was then completely macerated. The solution was filtered through paper and the filtrate, having been raised to body temperature, was injected intravenously.

We have never found it necessary to cut down on a vein under general or local anesthetic. The skin surface above the vein is shaved and the needle inserted into the vein which is dilated by compression. The dogs make very little resistance to this procedure and after the first few punctures lie quietly during the operation. Sugar injections (100 c.c. of fluid) as well as the numerous administrations of extract were conducted by this method.

We performed several experiments with the object of exhausting the zymogen granules of the pancreas. Prolonged secretin injections and vagus stimulation below the diaphragm were practiced. Fortune favored us in the first experiment. In subsequent attempts we were never able to exhaust the gland sufficiently to obtain an extract free from the disturbing effects of some constituent of pancreatic juice.

The blood sugar estimations were made by the Myers-Bailey[21] modification of the Lewis-Benedict method. The results of this method were corroborated by the Schaffer-Hartman[22] method at high and low percentages of blood sugar. The former method gave results which were consistently slightly higher (.01 per cent) than those obtained by the Schaffer-Hartman method. We find the average normal blood sugar, from observations on thirty normal dogs, to be .090 per cent.

Hemoglobin estimations were made by the carbon-monoxide saturation method, using the du Bosecq colorimeter.

RESULTS

Chart 1 contains the record of a 6.5 kilogram dog (410). This experiment is not conclusive but is interesting to us at least, since we administered the first dose of extract of degenerated pancreas to this animal. On July 11, the pancreas, with the exception of the processus uncinatus, was removed. The processus was allowed to remain until July 18. In the interval between the operations there was no hyperglycemia or glucosuria. The curves on subsequent days show the effect produced by the removal of the pedicle. It will be noted that as the experiment progresses the percentage of blood sugar did not rise to the level usually attained in completely depancreatized animals, and also that there was a marked decrease in the daily amounts of nitrogen and sugar excreted and the volume of urine voided. The animal continued to lose weight and seemed to be entering the cachexial condition characteristic of depancreatized animals which had become infected.

The chart for July 27 shows the effect produced on the percentage of

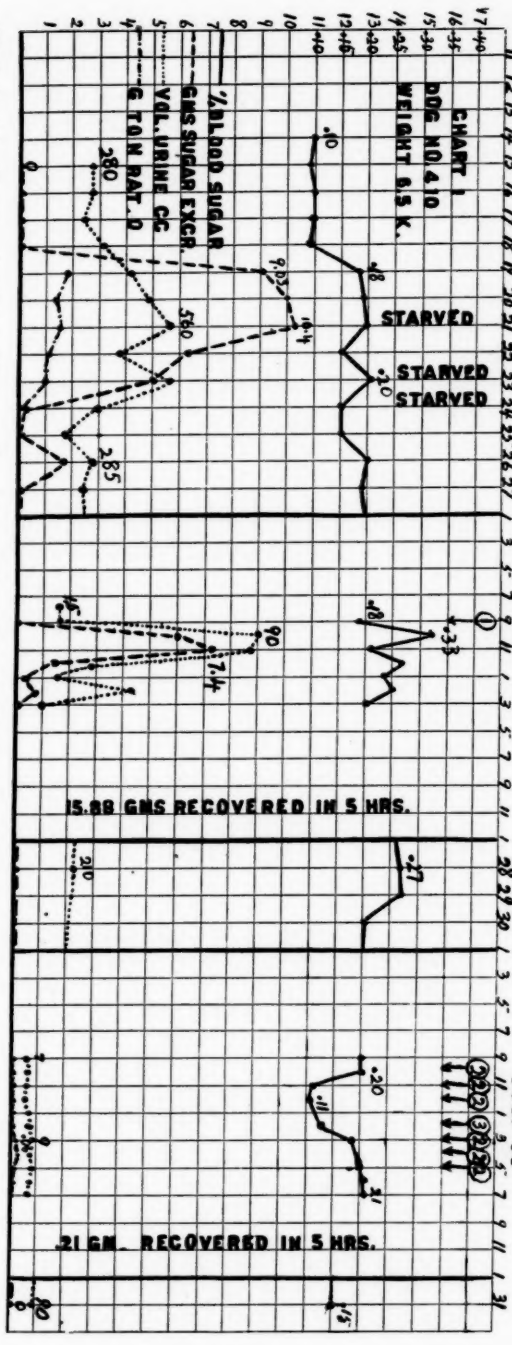

Chart 1.—(1) 25 gms. glucose in 250 c.c. water by stomach tube. (2) Degenerated gland extract, 5 c.c. dose intravenously. (3) .20 gms. glucose in 200 c.c. water by stomach tube. Dog died July 31st—cachexia.

blood sugar and on the sugar excretion by the oral administration of twenty-five grams of dextrose in two hundred and fifty c.c. of water.

At 10 A.M. July 30, the percentage of blood sugar was 0.20. Four c.c. of extract of degenerated pancreas were injected intravenously. At 11 A.M. the blood sugar had fallen to 0.12 per cent. The injections of extract are shown

in the chart. At 12 A.M. twenty grams of sugar in two hundred c.c. of water were given by stomach tube. The chart records the effect.

The obvious criticism of this experiment is that the animal was moribund when the effect of the extract was tried. The interesting features, which gave us great encouragement are (1) the extract caused a sudden fall in the blood sugar and (2) that in the presence of the extract the animal excreted .21 grams of a 20 gm. injection in a period of five hours following the injection, in contrast to an excretion of 15.88 grams of a 25 gm. injection in the same interval, when no extract was administered.

Chart 2 is the record of dog 92, weight 11.9 kg. A complete pancreatectomy was performed on this animal at three P.M. August 11. The first injection of extract was given six hours after the operation and subsequently an injection every four hours. This extract was freshly prepared from a ten Kg. dog whose pancreatic ducts had been ligated for ten weeks. One hundred and twenty-five c.c. of extract were prepared from the gland residue but this supply was exhausted by two P.M., August 13, after which other extracts were used. Blood samples were always taken before the injections of extract.

On August 12, the blood sugar curve shows that neither five nor eight c.c. of this extract every four hours were sufficient to counterbalance the upward trend of the percentage of sugar of the blood. At 10 P. M. the dose was increased to twelve c.c. and a marked fall is noted. The chart at 10 A. M. August 14 records the reduction of the percentage of sugar in the blood below its normal level, as a result of extract from another degenerated gland. (The exceptionally higher values for the volume of urine and the urinary nitrogen for August 15 and 16 may be due to the adulteration of the urine with vomit.) On August 15 at 10 A. M. the chart shows the effect produced by ten c.c. of the same gland extract made 0.1 per cent acid with HCl. This extract made 0.1 per cent alkaline with NaOH causes a slight reduction (August 15, 8 P. M.) The effect may be due to the alkali.

The extract administered at 10 A. M. August 16 was neutral and made from the same degenerated gland.

On August 16 and 17 effects of extracts from normal glands were tested. A normal pancreas from a ten kg. animal was divided into three equal parts. One third was extracted with neutral saline, the second portion with 0.1 per cent HCl and the third with 0.1 per cent NaOH. On August 17 at four P. M. the neutral whole gland extract was administered. A marked fall in blood sugar resulted. The acid and alkaline extracts were injected at 12 P. M. August 17, and 7 A. M. August 18. The last two injections were perhaps not given a fair opportunity to develop their effects. We do not take colorimeter readings by artificial light and therefore did not have an accurate knowledge of the height of blood sugar at these times.

The conclusion from this experiment is that freshly prepared neutral or acid extracts of the whole pancreas do have a reducing effect on blood sugar, thus confirming Kleiner. It may be stated here that repeated injections of whole gland extracts cause marked thrombosis of the veins where the injections are made and a noticeable interference with kidney function. It is obvious from the chart that the whole gland extract is much weaker than that from the degenerated gland.

On August 20, we attempted to exhaust the pancreas of a nineteen kg. dog by continued injections of secretin and repeated stimulation of the vagus nerve below the diaphragm. We obtained eighty-five c.c. of pancreatic juice and considered the gland exhausted. It was swiftly removed and immediately chilled. The marked effect of injection of this material is shown on the chart at 7 P. M. August 20. On August 21 we incubated ten c.c. of the extract and five c.c. of pancreatic juice for two hours at

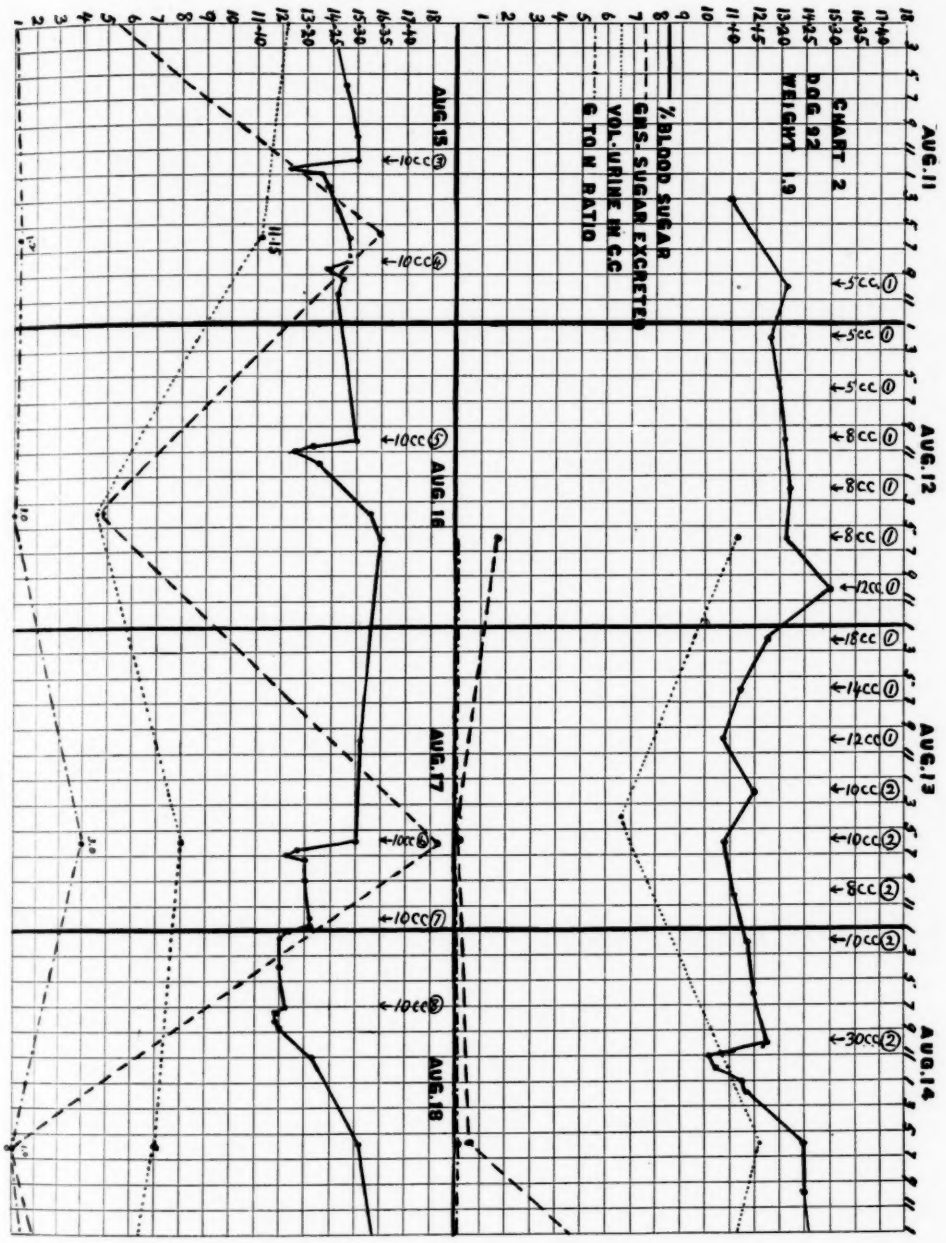

Chart 2.—(1) Degenerated pancreas, dog 394. (2) Degenerated pancreas, dog 390. (3) Degenerated pancreas, + .1% HCl. (4) Degenerated pancreas + 0.10% NaOH. (5) Degenerated pancreas, + .1% HCl. (6) Whole gland extract, fresh, cold. (1) Whole gland extract, + .1% HCl. (8) Whole gland extract + .1% NaOH. (9) Exhausted gland extract. (10) 10 c.c. exhausted gland extract + 5 c.c. pancreatic juice incubated 2 hours. (11) 10 c.c. exhausted gland extract (– pancreatic juice) incubated 2 hours. (12) Whole gland, cat. Dog died August 30.

body temperature in alkaline solution. This solution was injected at 6 P. M. August 21. The curve shows the very slight effect produced. As a control on the above, ten c.c. of extract and five c.c. of saline were incubated under similar conditions for two hours. The chart at 10 P. M. August 21 records the marked effect of the injection of this second solution. On August 22 at 6 P. M. eight c.c. of extract from the normal

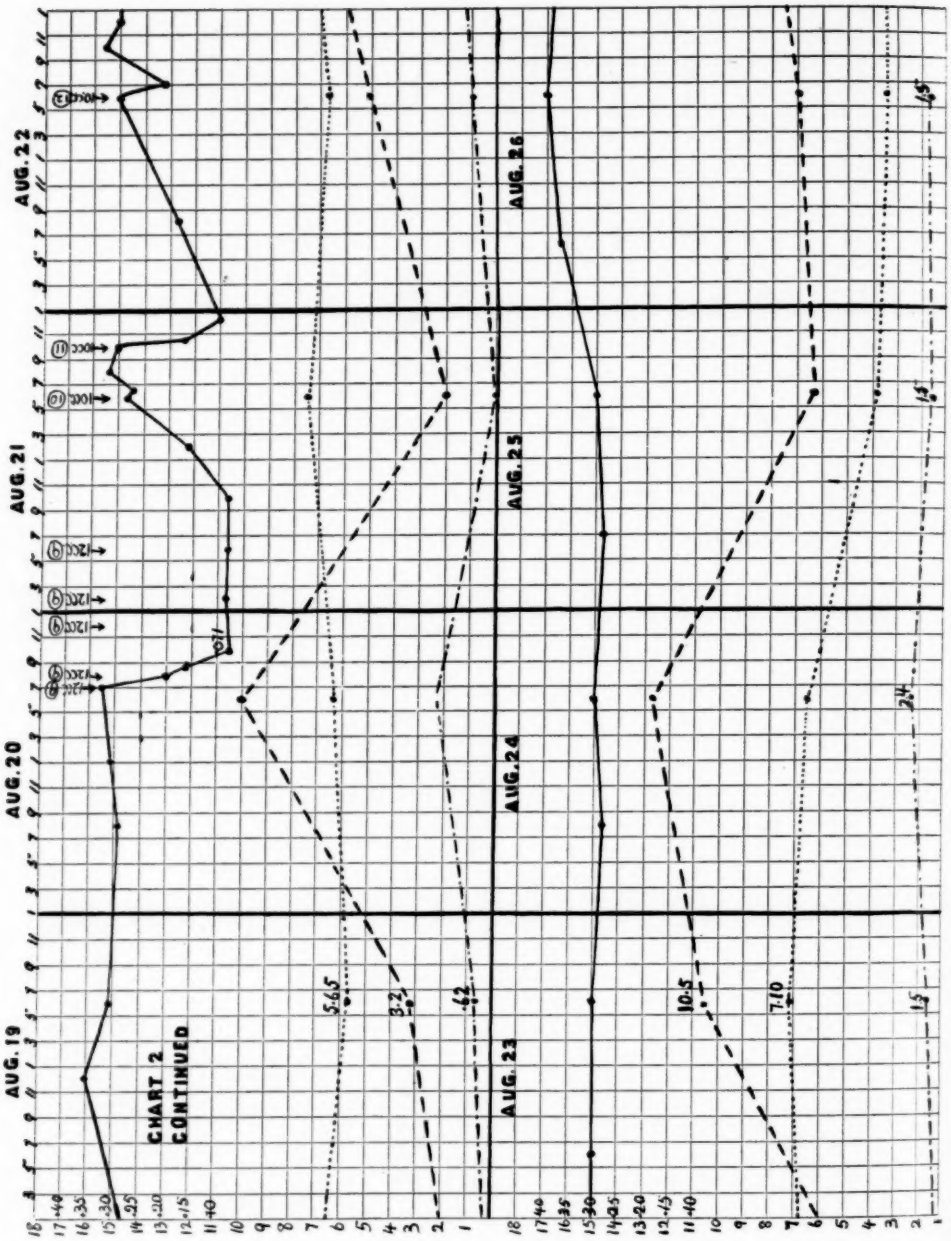

Chart 2.—Continued

pancreas of a cat were injected. We obtained a marked anaphylactic-like reaction. The curve shows the effect upon the blood sugar.

No further injections were given to this animal after August 22. The dog died on August 30, nineteen days after the operation. The autopsy showed consolidation and necrosis of a large area in lower lobe of right lung, infection in right pleural sac. The operation wound was well healed. There was no sign of pancreatic tissue. The abdomen was not infected.

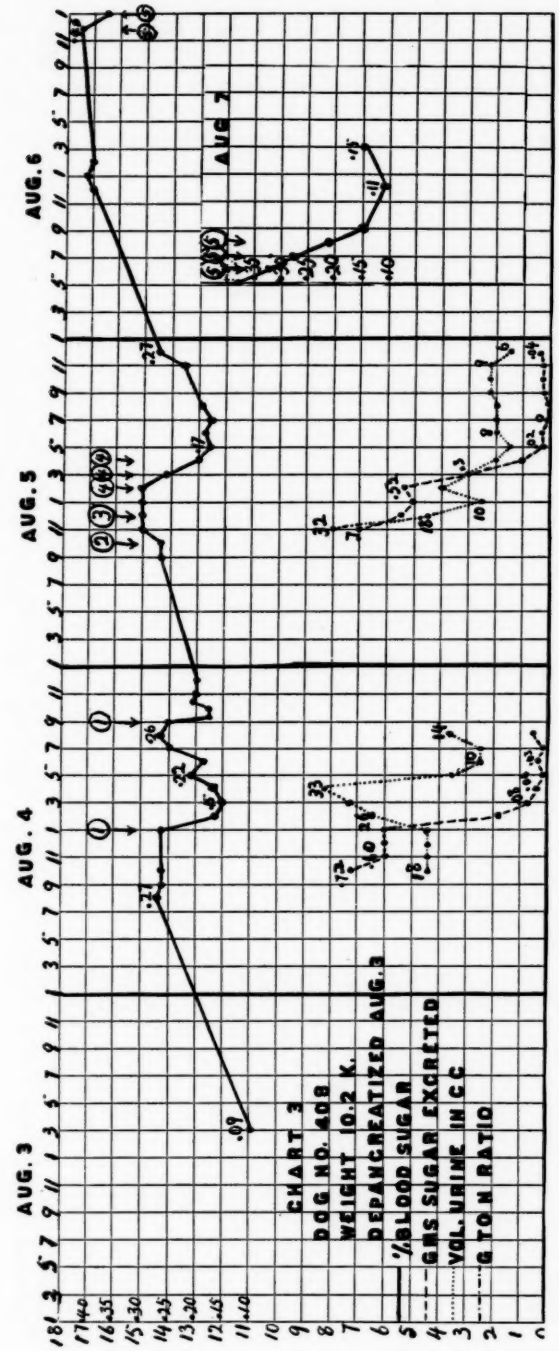

Chart 3.—(1) 5 c.c. 4 day old extract of degenerated pancreas. (2) 5 c.c. extract of liver. (3) 5 c.c. extract of spleen. (4) 5 c.c. extract of degenerated pancreas. Dog died August 7—general peritonitis.

Chart 3 is the record of dog 408. The weight of this animal was 9 kg. The details of the experiment will be given rather fully.

The normal blood sugar of dog 408 was .090 per cent. Eighteen hours after pancreatectomy the percentage of sugar in the blood was .27. Twenty-two hours after

the operation, 1 P. M. August 4, the blood sugar was .26 per cent. During the twenty-two hours 3.10 grams of sugar were excreted. The volume of urine was 494 c.c. At one P. M. we administered five c.c. of extract of degenerated pancreas which had been prepared four days previously and kept in cold storage. At two P. M. the blood sugar was .16 per cent. At three P. M. the percentage of sugar in the blood had fallen to .15. From one to three P. M. .19 grams of sugar were excreted in a volume of twenty-

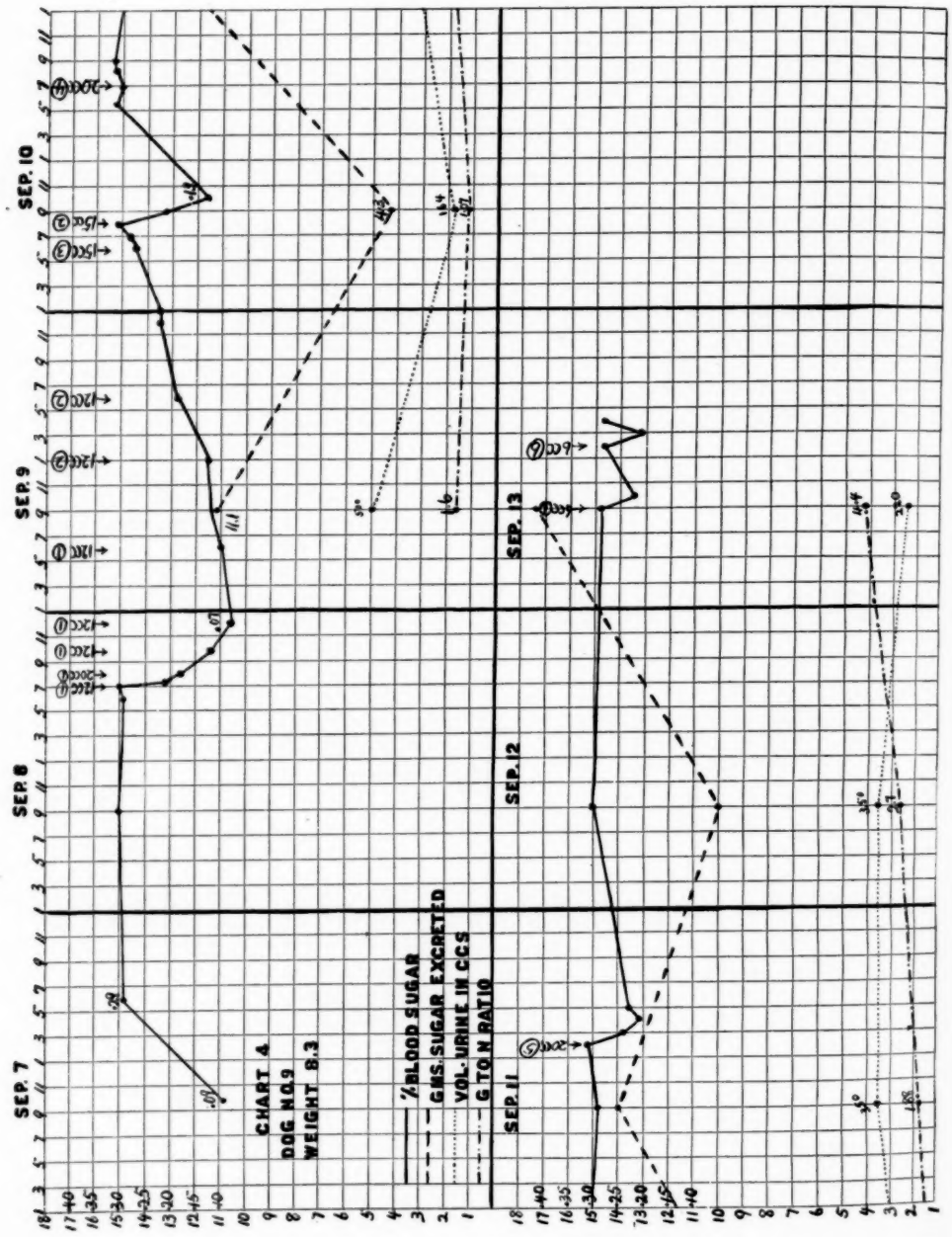

Chart 4.—(1) Exhausted gland extract 1% HCl. (2) Exhausted gland extract neutralized. (3) Exhausted gland extract neutralized per rectum. (4) 20 c.c. exhausted gland extract and alkali + 10 c.c. pancreatic juice incubated 3 hours at 37°C. then neutralized. (5) 20 c.c. exhausted gland extract incubated 3 hours at 37° C., 0.1% HCl. (6) Exhausted gland of cat, 0.2% HCl.

six c.c. of urine. Three and forty-three hundredths grams of urinary nitrogen were excreted in the twenty-four hours following the operation. The G. to N. ratio for this period was 1.4:1. From 3 P. M. to 7 P. M. the percentage of sugar in the blood shows a gradual rise from .15 to .25 per cent. This latter level was maintained until 9 P. M. The chart shows a slight rise in sugar excretion following the rise of blood sugar. At 9 P. M. five c.c. of extract which had been exposed to room temperature for one hour was injected intravenously. The blood sugar was reduced to a value of .18 per cent. The chart shows a gradual ascent from this value to .27 per cent. At 10 P. M. the percentage of sugar in the blood was .27. At this hour five c.c. of extract of liver, prepared in precisely the same manner as the pancreatic extract, were administered intravenously. One hour later the blood sugar was .30 per cent. This level was maintained during the following three hours. It was unaffected by an injection of 5 c.c. of extract of spleen. The chart shows the rise in volume of urine and amount of sugar excreted. At 2 P. M. (b. s. .3 per cent), five c.c. of an extract of degenerated pancreas were injected. A sharp fall in the blood sugar resulted. At 3 P. M. and again at 4 P. M. a similar dose of extract was given. The chart records the lasting effect. The 2 P. M. level of .30 per cent was regained twelve hours after the first injection. The hourly excretion of sugar ran approximately parallel with the percentage of sugar in the blood. Between 1 P. M. and 2 P. M. .52 grams were excreted. Less than .02 grams were excreted between 7 P. M. and 8 P. M. The highest glucose to nitrogen ratio observed in this experiment was a 3:1 value for the 22-hour interval between 2 P. M. the 6th of August and noon the following day. At 12 noon August 6 the percentage sugar in the blood was .40 per cent. Five c.c. of boiled extract of degenerated pancreas was injected intravenously at this stage and caused no reduction of blood sugar. At twelve midnight August 6 five c.c. of extract of degenerated pancreas which had been prepared 48 hrs. previously were administered. The blood sugar fell from .43 per cent at 12 P. M. to .37 per cent at 1 A. M. Five c.c. doses were given at 1, 2 and 3 A. M. and a twenty-five c.c. dose at 4 A. M. The chart shows the reduction in blood sugar to a normal level and the beginning of an upward trend five hours after the last injection of extract. The animal died at 12 A. M. August 7.

A brief description of the clinical condition of the animal at various stages of the experiment is necessary for the correct interpretation of the above results. The animal made a good postoperative recovery and was able to retain water and meat after the second day following the operation. On the morning of August 5 we noticed that the condition of the animal was much worse. It appeared excessively tired, did not eat, and vomited after drinking water and also after extract of spleen given intravenously. At 5 P. M. August 5 the animal appeared considerably improved. It retained water and ate meat. On August 6 at 10 P. M. the abdominal wound was moist with exudate, and the animal was not so active as on the preceding day. No marked variation from this condition was observed until 4 A. M. when 25 c.c. of extract were administered. After this injection the animal had a marked reaction and appeared to be dying. It was revived slightly by intravenous and intraperitoneal injections of warm saline. Considerable improvement was noted at 7 A. M., dog was able to stand. The improvement was short-lived. The dog died at 12 A. M. August 7. The postmortem showed a widespread abdominal infection. There was no sign of pancreatic tissue.

The entire degenerated pancreas from one 8 kg. dog and approximately one-half the degenerated gland from a 6 kg. dog was the substrate of the extract used in this experiment.

Chart 4, Dog 9, gives additional evidence on several important points which have been referred to previously. At 6 P.M., September 8, we administered ten c.c. of extract of degenerated pancreas *per rectum*. There was no reduction in blood sugar at 7 P.M. when we gave 12 c.c. of extract of exhausted gland intravenously. The chart records the effect of this and sub-

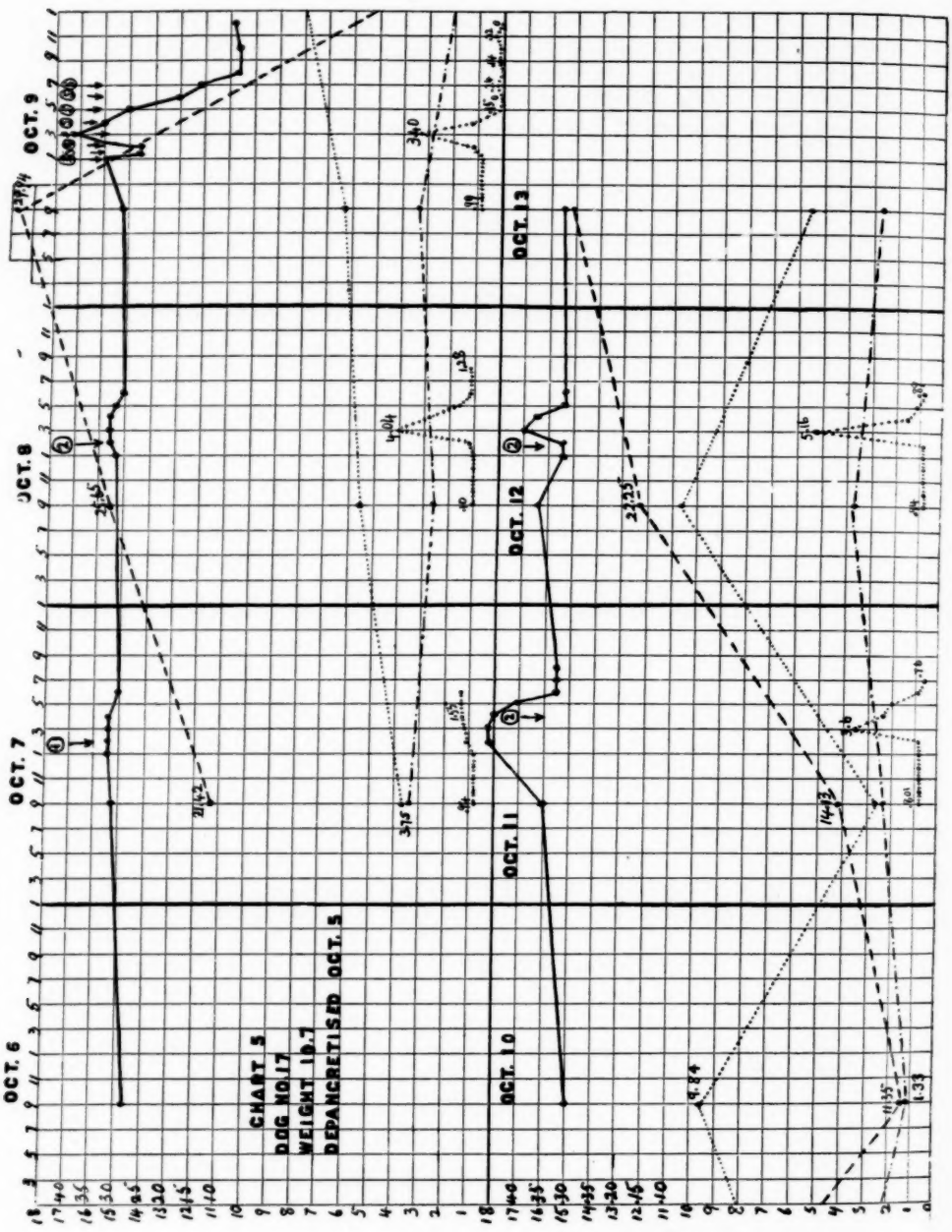

Chart 5.—(1) 100 c.c. saline. (2) 10 gms. sugar + 100 c.c. water. (3) 10 c.c. extract A. (4) 10 gms. sugar, 80 c.c. water, 20 c.c. extract A. (5) 20 c.c. extract B. *Note*: Extract A was made from uncinate process. Extract B from tail portion of pancreas.

sequent injections of the same material. At 6 A.M., September 10, we administered 15 c.c. of extract of exhausted gland per rectum. There was no effect. At 8 A.M., September 10, fifteen c.c. of extract of exhausted gland were injected intravenously. The drop in blood sugar was very marked. Twenty c.c. of exhausted gland extract, made 1 per cent alkaline with NaOH, were

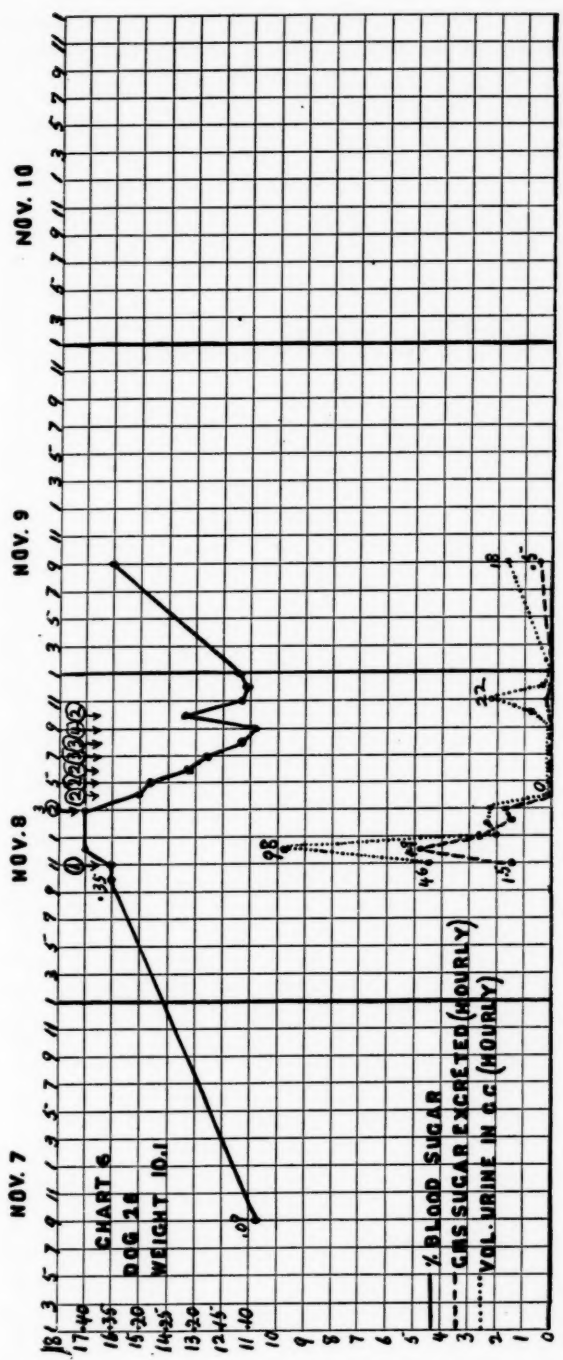

Chart 6.—(1) 10 gms. sugar in 100 c.c. water. (2) 15 c.c. extract degenerated pancreas (4 weeks after ligation of ducts). (3) 6 c.c. extract degenerated pancreas (7 weeks after ligation of ducts). (4) 10 gms. sugar in 100 c.c. water. Dog died November 10—duodenal ulcer.

incubated three hours at body temperature with 10 c.c. of active pancreatic juice. This solution was neutralized and injected intravenously at 7 P.M. September 10. No reduction in blood sugar resulted. At 2 P.M. September 11, 20 c.c. of acid extract incubated for three hours at 37.5° F. were injected. The curve shows the drop in blood sugar. On September 13 at 9 A.M. and

2 P.M. the effect of extracts from the partially exhausted gland of a cat is shown. This extract produces a pronounced general reaction.

We observe that extracts prepared from these more or less exhausted glands, while retaining to some extent the reducing effect upon blood and urine sugar, produce many symptoms of toxicity which are absent after injections of extracts from completely degenerated glands.

Chart 5 is the graphic record of an experiment on a 10 Kg. dog in which we have attempted to prove that the reduction of the percentage sugar in the blood is not a dilution phenomenon. Our plan of campaign was to inject at a given hour on the first day (2 P.M., October 7), 100 c.c. of isotonic saline (*1*). On the second day at the same hour the animal received 100 c.c. of 10 per cent sugar solution (*2*). Extract (*3*) (10 and 15 c.c. doses) was given one hour, and a second dose thirty minutes before the corresponding hour on the third day and 70 c.c. of distilled water containing 10 gms. of sugar were injected on the hour (*4*). Extract in 20 c.c. doses was injected at 3, 4, 5, 6 and 7 P.M. (*5*). To interpret correctly the curve for this day, a brief description of extracts (*3*) and (*5*) is necessary. The pancreas from which these extracts were made was not completely degenerated. The pancreatic ducts of the animal had been tied six weeks. Extract (*3*) was made from the processus uncinatus. Extract (*5*) was made from the remainder of the gland. The extract from the uncinate process was much weaker than the latter. In the four hours following the first injection of sugar we recovered 9.94 grams in the urine. In the corresponding period on the following day after the injection of sugar plus extract 4.49 grams were recovered. Had we used the more powerful extract first, the reducing action might have been even more strikingly demonstrated.

We were surprised that we did not secure a raised blood sugar one hour after the first injection of sugar. The rise after the second injection was very marked. We gave the animal one day's rest and repeated the consecutive injections with the same results as above. The interpretation of the results of the first of the later injections is complicated by an unexplainably high percentage of sugar present before the injection. This phenomenon cannot be wholly explained by the rate of output of sugar since on the fourth injection, the first hour excretion was the maximum of the series and we did obtain a definite rise in blood sugar. An injection of 1 gram of sugar per kg. given to a normal dog showed a pronounced rise in percentage sugar of the blood after a 15-minute interval. This hyperglycemia rapidly subsided and at the end of an hour the blood sugar had regained its normal level; 2.29 grams of sugar were excreted.

Hemoglobin estimations were made (1) one hour after the first sugar injection, (2) just before the first injection of extract and (3) at 12 P.M., Oct. 9. The blood sugars at these times were .33 per cent, .35 per cent and .09 per cent respectively. The hemoglobin was identical at the second and third determinations; a slightly lower value was obtained at the first determination.

Chart 6 is the record of a short, but very interesting experiment which again demonstrates the remarkable effect of the extract of degenerated pan-

creas upon the power of a diabetic animal to retain sugar. On Nov. 8 at 11 A.M. (b.s. 35 per cent), 10 gm. of sugar were injected intravenously. The curve shows the rise in blood sugar. In the four hours following the injection, 10.88 gm. of sugar were excreted. From 3 to 9 P.M. 78 c.c. of dilute extract were injected in 13 c.c. doses. At 9 P.M. (b.s. .09 per cent), 10 gm. of sugar were injected. The curve shows the effect on blood sugar and sugar excretion. The effect of partially degenerated gland extract, 5 weeks after ligation of the ducts, upon the kidneys is shown here. This extract may produce a raised threshold to sugar or a condition of anuria, as in this experiment. Hemoglobin estimations before and after administration of extract were identical. Duodenal ulcer was the cause of the early termination of the experiment.

A more detailed description of the histologic sections obtained during our experiments will be included in a subsequent communication. Suffice it here to note that the pancreatic tissue removed after seven to ten weeks' degeneration shows an abundance of healthy islets, and a complete replacement of the acini with fibrous tissue.

In the course of our experiments we have administered over seventy-five doses of extract from degenerated pancreatic tissue to ten different diabetic animals. Since the extract has always produced a reduction of the percentage sugar of the blood and of the sugar excreted in the urine, we feel justified in stating that this extract contains the internal secretion of the pancreas. Some of our more recent experiments, which are not yet completed, give, in addition to still more conclusive evidence regarding the sugar retaining power of diabetic animals treated with extract, some interesting facts regarding the chemical nature of the active principle of the internal secretion. These results, together with a study of the respiratory exchange in diabetic animals before and after administration of extract, will be reported in a subsequent communication.

We have always observed a distinct improvement in the clinical condition of diabetic dogs after administration of extract of degenerated pancreas, but it is very obvious that the results of our experimental work, as reported in this paper do not at present justify the therapeutic administration of degenerated gland extracts to cases of diabetes mellitus in the clinic.

CONCLUSIONS

The results of the experimental work reported in this article may be summarized as follows:

Intravenous injections of extract from dog's pancreas, removed from seven to ten weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine.

Rectal injections are not effective.

The extent and duration of the reduction varies directly with the amount of extract injected.

Pancreatic juice destroys the active principle of the extract.

That the reducing action is not a dilution phenomenon is indicated by the

following facts (1) hemoglobin estimations before and after administration of extract are identical; (2) injections of large quantities of saline do not effect the blood sugar; (3) similar quantities of extracts of other tissues do not cause a reduction of blood sugar.

Extract made 0.1 per cent acid is effectual in lowering the blood sugar.

The presence of extract enables a diabetic animal to retain a much greater percentage of injected sugar than it would otherwise.

Extract prepared in neutral saline and kept in cold storage retains its potency for at least seven days.

Boiled extract has no effect on the reduction of blood sugar.

We wish to express our gratitude to Professor Macleod for helpful suggestions and laboratory facilities and to Professor V. E. Henderson for his interest and support.

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THE EFFECTS OF VARIOUS FOODS, FOOD FACTORS AND CHEMICAL AGENTS UPON THE RESISTANCE OF ANIMALS TO ACETONITRILE*

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THE correlation between the state of nutrition of animals and their resistance to poisons has claimed the attention of many investigators. La Monaco and Trambusti,[1] (1894) reported a difference in the toxicity of phosphorus in well nourished animals and in poorly fed ones. Foster[2] (1910) studied the resistance of dogs to ricin and artificial hemorrhage on various planes of protein intake but found no difference. Hunt[3] (1910) observed an increase in resistance in mice to acetonitrile when on a restricted diet. Ellinger[4] (1905) reported the failure of cantharides to produce injury to the kidneys of rabbits fed on carrots. Hunt noted striking alterations in the resistance of rats, mice and guinea pigs to poison, following a change in their diets. The bibliography on this general subject is extensive and varied and cannot be extended here.

Our ideas of what constitutes a food have changed decidedly in the past few years. One of the most notable advances relates to the importance of the quality of protein furnished in the food. Not only must proteins constitute a part of every diet, but they must be of a suitable amino acid make-up. In other words, the indispensability of certain amino acids in the diet has been emphasized. Again, the need of the mineral elements in the diet has been studied with the result that the relative importance of several of them is now better recognized. The knowledge of the vitamins, recently discovered and so widely studied at the present time, has revolutionized experimental feeding. Since an inadequate diet manifests its effects in altered metabolism, the influence of controlled intake, both qualitatively and quantitatively, in investigations upon the action of poisons in the body is of prime importance. It seemed desirable, therefore, to repeat some of the experiments on the effects of poisons in the light of the newer advances in the science of nutrition.

Hunt has shown that acetonitrile is a poison in the animal body and has stated that it acts as a very delicate test for certain changes in metabolism. The plan of the present experiments was to use as a basal diet a food mixture qualitatively and calorifically adequate, and to study the variation in resistance of mice to acetonitrile when the proportion of various constituents of the diet was varied as well as when other substances were added to the diet.

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EXPERIMENTAL PART

The basal diet was made up as follows: Casein 31.0, butter fat 21.6, brewery yeast 2.0, salt mixture* 6.8, and cornstarch 38.6 per cent.

The protein and starch had been extracted with alcohol to free them from accessory food substances. That this diet was adequate for maintenance or slight gain was shown by the fact that we have kept adult animals on it for seven months in good condition. In the tests of toxicity Kahlbaum's acetonitrile in distilled water was injected subcutaneously. Controls were used in each series.

As numerous experiments were performed, only the maximum nonfatal and the minimum fatal doses are given from one selected series of these repeated experiments in the following tables:

TABLE I

	BASAL DIET	DEFICIENCY IN WATER SOLUBLE VITAMIN B	DEFICIENCY IN FAT SOLUBLE VITAMIN A	DEFICIENCY IN BOTH VITAMINS	UNDERFEEDING
Change in Body Weight, gm.	17.8-20.2 (+12%)	18.3-14.1 (−24%)	17.9-17.0 (−5%)	18.5-14.4 (−23%)	18.3-16.6 (−10%)
Duration of Feeding, days	22-28	22-28	22-28	22-28	22-28
Number of Mice Tested	9	9	9	9	9
Acetonitrile (mg. per gm. mouse)					
Max. Nonfatal	0.23	0.28	0.22	0.28	0.26
Min. Fatal	0.23	0.20	0.23	0.25	0.25

EFFECT OF LACK OF VITAMIN

Mice fed upon the above mixture without yeast, i.e., on a diet deficient in vitamin B, lost weight after an initial maintenance or slight gain. Though the effect of deficiency in vitamin A, secured by replacing butter fat in the basal diet with lard, was slower in making itself evident, our results plainly indicate the indispensability of both vitamins to adult as well as to growing mice. Acetonitrile was injected at varying intervals in different mice after the decline in weight appeared. As seen from Table I deficiency or lack of one or both vitamins had no effect on the resistance of mice to poisoning with acetonitrile.

EFFECT OF UNDERFEEDING

It is often stated that temporary starvation has a beneficial effect in many diseases. Experiments with underfeeding were carried out in connection with acetonitrile tests similar to those of Hunt but with more accurately controlled food intake. When the experimental mice were fed one-fourth of the food eaten by the control mice they lost weight quite rapidly. To avoid such unduly severe loss in weight the mice were therefore given one-half the amount of food eaten by the control animals. Except in the case where rolled oats

*Osborne and Mendel: Jour. Biol. Chem., 1918, xxxiv, 131. The lactose was omitted.

and water alone were given, underfeeding did not decrease the toxicity of acetonitrile, unless the animals were fed anew small quantities of the food just a few hours before the injection. (See Table II.)

TABLE II

	LOW PROTEIN DIET*		SALT-POOR DIET		OATS DIET	
	CONTROL	UNDER-FEEDING	CONTROL	UNDER-FEEDING	CONTROL	UNDER-FEEDING
(A) ACETONITRILE INJECTION WITHOUT PREVIOUS FEEDING.						
Change in Body Weight, gm.	21.7-15.4 (−30%)	20.6-14.0 (−32%)	21.3-21.1 (−1%)	22.4-18.0 (−20%)	16.2-15.2 (−6%)	15.1-12.1 (−20%)
Duration of Feeding, days	11	11	11	11	11	11
Number of Mice	6	6	5	5	5	5
Acetonitrile						
Max. Nonfatal	0.32	0.32	0.38	0.28	0.40	0.64
Min. Fatal	0.30	0.30	0.40	0.32	0.44	0.70
(B) ACETONITRILE INJECTION AFTER PREVIOUS FEEDING.						
Change in Body Weight, gm.	23.1-23.5 (+1%)	23.2-19.8 (−14%)	14.1-14.4 (+2%)	14.3-12.4 (−13%)		
Duration of Feeding, days	10	10	10	10		
Number of Mice	6	7	6	7		
Acetonitrile						
Max. Nonfatal	0.35	0.45	0.12	0.25		
Min. Fatal	0.40	0.50	0.14	0.24		

* The composition of the diet is shown in Table III, footnote.

EFFECT OF RATE OF GROWTH

Hunt stated that in his experiments the greater the rate of growth the greater was the susceptibility of the animal to acetonitrile; whereas animals increasing in weight slowly, showed relatively greater resistance to it. However, in our experiments with two groups of young mice, one group growing faster than the other, no difference in the resistance to acetonitrile was observed.

EFFECT OF DIETS LOW IN PROTEIN

Salant and Rieger[5] (1917) reported that with diets low in protein, an increased resistance to the effects of caffein was observed. In one of our series of experiments we substituted carbohydrate isodynamically for protein, and fat for protein in another series. Hunt reported increased resistance when various carbohydrates were superimposed upon his cake diet.

TABLE III

	BASAL DIET	LOW PROTEIN (HIGH CARBOHYDRATE)*
Change in Body Weight, gm.	17.5-19.0 (+8%)	17.9-18.2 (+1%)
Duration of Feeding, days	14	20
Number of Mice	21	9
Acetonitrile		
Max. Nonfatal	0.40	0.48
Min. Fatal	0.40	0.50

*Casein 6.0, dextrin 25.0, butter fat 21.6, brewery yeast 2.0, salt mixture 6.8 and cornstarch 38.6 per cent.

TABLE IV

	BASAL DIET	LOW PROTEIN (HIGH FAT)**
Change in Body Weight, gm.	16.4-17.1(+3%)	16.4-13.1(-20%)
Duration of Feeding, days	12	12
Number of Mice	6	6
Acetonitrile		
Max. Nonfatal	0.56	0.40
Min. Fatal	0.60	0.40

**Casein 4.0, lard 13.5, butter fat 21.6, brewery yeast 2.0, salt mixture 6.8 and cornstarch 38.6 per cent.

As will be seen from Tables III and IV the low protein diet in which carbohydrate is substituted for some of the protein increased the resistance of the mice somewhat while the diet in which protein was largely replaced by fats decreased the resistance of the mice. A distinct tendency to lose weight was noticed in the fat experiments.

EFFECT OF DIETS HIGH IN FATS

Salant and Nelson (1917) found a modification of the toxicity of chenopodium oil in cats by feeding coconut and cottonseed oils. Hunt reported that a high fat diet rendered mice less resistant to acetonitrile. In the diet used in the present experiment a large part of the carbohydrate was replaced isodynamically with lard. As seen from Table V our results show that on a diet high in fat mice are slightly less resistant to acetonitrile. We have found this true especially after a long period of eating such a diet.

TABLE V

	BASAL DIET	FAT DIET*
Change in Body Weight, gm.	25.5-27.5 (+8%)	24.9-27.3 (+9%)
Duration of Feeding, days	33	33
Number of Mice	5	5
Acetonitrile		
Max. Nonfatal	0.32	0.22
Min. Fatal	0.34	0.24

*Casein 25.0, butter fat 21.6, lard 18.0, brewery yeast 2.0, salt mixture 6.8 and cornstarch 8.6 per cent.

EFFECT OF KIND OF FAT

Since the character of the fat deposited in the body is dependent, to some extent, upon the nature of the fat fed, and since various fats may conceivably be oxidized with different facility, it seemed of interest to try the effect of fats of various kinds to see whether or not the ease of fat oxidation in the

TABLE VI

	BASAL DIET	COD LIVER OIL DIET*	OLIVE OIL DIET*
Change in Body Weight, gm.	17.9-19.8 (+10%)	18.0-18.2 (+1%)	17.8-17.8 (±0%)
Duration of Feeding, days	14	14	14
Number of Mice	7	8	8
Acetonitrile			
Max. Nonfatal	0.60	0.40	0.40
Min. Fatal	0.55	0.45	0.45

*Two-thirds the amount of butter fat in the basal diet were replaced with oils under investigation.

body had any influence upon the simultaneous oxidation of acetonitrile. Butter fat, olive oil and cod-liver oil were the fats used. From Table VI it is seen that olive oil and cod-liver oil tend to lower the resistance slightly.

EFFECT OF VARIOUS ANIMAL TISSUES

Since many of the organs are more or less closely associated with growth, nutrition and immunity, the effect of feeding various organs on the resistance to acetonitrile was tested. Hunt has reported the effects with various tissues but the result with thyroid was most striking. In the present experiments tissues were fed in small quantities varying from 0.1 to 3.0 gms. of dried tissue-substance in 100.0 gms. of basal diet and replacing an amount of protein calculated from the factor $N \times 6.25$. The tissues were kidney, pancreas, submaxillary, parotid, ovary, lymph gland, suprarenal, brain, heart muscle, skeletal muscle, and pituitary. None of these had any effect on the resistance to acetonitrile except kidney which seemed to lower it. When preparations from fresh lamb kidney and beef liver were fed, no effect on the resistance was observed. In our trials with tissues added to the diet, there have been no instances of increased resistance to acetonitrile except in the case of the thyroid which will be discussed elsewhere.

EFFECT OF MINERAL ELEMENTS

The literature on salt metabolism and mineral requirements in nutrition is too extensive to review here. The problem of salt action in the body, the rôle of salts in absorption and osmotic phenomena, the part played by salt ions in conduction and irritability and the importance of salts in the acid-base equilibrium in the blood add importance to the physiology of mineral elements in the body. In the present experiments, diets poor in Ca, Cl, SO_4, PO_4, K, Mg, and Na, respectively, were used. These various salt mixtures were prepared, exactly following the plan of Osborne and Mendel (1918) for studies of inorganic elements in nutrition. In addition, diets poor in salts,* free from iodine and with iodine alone were used. The results with food lacking one or other of the elements were so variable that they were insignificant. With the salt-poor and iodine-free diets, the resistance to acetonitrile was diminished while with the iodine diet loss of resistance was less. Hunt also found that iodine seemed to impart some variable degree of resistance to the poison.

EFFECT OF KIND OF PROTEIN

Since some of the various amino-acids are structurally similar to active components of some of the internal secretions, and since it seems that in its toxicity in the animal body acetonitrile involves processes governed by these internal secretions, at least in part, it seemed important to study the effect of variation in the composition of the proteins making up the diet. Gelatin, casein and edestin were tested. Tyrosine was used to supplement the gelatin and in some of the experiments, casein and gelatin were fed together. When gelatin and gelatin-tyrosine were the sole source of protein in the diet there

*Salt mixture was omitted from the basal diet.

was an increase in resistance to acetonitrile. When edestin or gelatin-casein were the source of protein there was no increase in the resistance to the poison. Hunt reported experiments which ours seem to corroborate with protein in general.

EFFECT OF OAT STARCH

According to Hunt, mice fed upon oatmeal or oats exhibit an increase in resistance to acetonitrile. He attributed this beneficial effect to the increased ability of the thyroid to take up iodine after feeding with oats.

In the present experiments, oat starch was prepared from rolled oats. Mice fed with the oat starch diet showed a remarkable resistance to acetonitrile. When, however, the starch was first extracted with alcohol and ether, there was no increase in the resistance to the poison. The ash of the oats likewise had no effect in altering the susceptibility to the poison.

CONCLUSION

The foregoing experiments seemed warranted from the standpoint of the use of improved technic. We have employed standardized, synthetic foods, the nutritional significance of every factor of which is known. This has enabled us to vary the constituents of the food at will while keeping the composition of the food under exact control. The experimental evidence shows that only in the cases of underfeeding, of feeding with iodine as the sole mineral, of feeding with oat starch and possibly of feeding with diets high in fats, was any alteration in the resistance to acetonitrile secured.

It appears therefore that the susceptibility to poisoning in mice with acetonitrile is not easily affected by changes in dietary composition within wide limits of quantity and especially of quality. Perhaps this may serve to emphasize the factor of safety possessed by the organism in its response to alterations of the diet.

The author desires to express his thanks to Professor Lafayette B. Mendel for his helpful advice and criticism.

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EFFECT OF TIME BETWEEN OBTAINING A SPINAL FLUID AND MAKING A CELL COUNT ON THE RESULT OF THE COUNT*

By James Wynn, M.D., Boston, Mass.

MOST writers in discussing the Fuchs-Rosenthal counting chamber and cerebrospinal fluid cell counting lead one to the inference that cells must be counted immediately after lumbar puncture if the count is to be relied upon. Boyd,[1] in referring to the Fuchs-Rosenthal method, mentions as a great practical advantage that it is usable at the bedside. Then follows the statement: "Indeed, the sooner the count is made after the withdrawal of fluid the better, as sedimentation occurs rapidly and the cells are liable to adhere to the sides of the tube." Kolmer[2] and others[3,4] have also specified that the fluids for counting must be fresh.

This is obviously true enough in most cases in which there is a very high degree of meningeal irritation. It is a matter of common experience for such fluids to show webs even before they can be rushed to the laboratory. Evidence, however, seems lacking to prove immediate counting necessary in case of normal spinal fluids or those with more moderate pleocytoses such as are characteristic of a large group of tabetics and neurosyphilitics.

In view of this scarcity of data, the cells in eighty spinal fluids from the wards of this hospital were counted at varying intervals (see Table I) up to fifteen hours after lumbar puncture. The fluids were collected (6 c.c. to the tube) in clean (not sterile) test tubes (10 × 100 mm.) and these were tightly stoppered with corks. Before each count the tubes were gently inverted twenty to thirty times. A Levy double counting chamber was used and the cells in eighteen square millimeters of fluid counted in each case. (Slight variations in the treatment of the fluid immediately before counting are explained in the legend of Table I.) During the intervals between counts, the first forty fluids of the series were placed in the ice box; the remainder were allowed to remain at room temperature. The counts are tabulated in Table I.

The constant association of macroscopic changes in the fluid with subsequent variations from the bedside cell count would seem to suggest that purely mechanical factors are responsible for such changes in count, and at Dr. Christian's suggestion it was decided to mix in a small quantity of powdered sodium citrate with one of the specimens collected in every case presenting on puncture any gross abnormality in the fluid, in order to see whether clotting might not be an important factor in causing the change in the cases and to see whether preventing the clotting might not prevent the occurrence of the change. Unfortunately, there have been only two such

*From the Medical Service of the Peter Bent Brigham Hospital, Boston, Mass.

TABLE I

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOBULIN	MACROSCOPIC APPEARANCE IMMEDIATELY	MACROSCOPIC APPEARANCE ON STANDING	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
L.A.S.	syphilis of c. n. s.	0.2 c.c. ++	++	clear	clear	A-3 P-3		A-3 P-4				A-3 P-3	
L.F.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-17 P-18		A-16 P-17				A-15 P-19	
R.P.	encephalitis lethargica	2.0 c.c. +±	–	clear	clear	A-270 P-276			A-280 P-178			A-281 P-280	
W.G.	encephalitis lethargica	1.0 c.c. +± 1.5 c.c. –	+	clear	clear	A-89 P-89			A-87				A-89
M.Z.	syphilitic meningitis (treated)	0.2 c.c. ++	++	clear	clear	O-10 5-12 sp10		O-9 5-12 sp11					O-11 5-13 sp11
M.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	O-33 5-29		O-35 5-21				O-34 5-31	
R.B.	tabes dorsalis	0.3 c.c. ++	++	clear	clear	5-126 P-126			5-128				5-124
K.L.	acute anterior poliomyelitis	2.0 c.c –	+±	clear	hazy in 5 hr.	P-148 A-146				P-132 A-124			
R.E.L.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	P-19 A-18			P-18 A-17			P-17 A-17	
R.C.	tabes dorsalis	0.7 c.c. ++	++	clear	clear	P-34 A-34		A-31			A-32		
J.L.	encephalitis lethargica	2.0 c.c. –	++	clear	sed. in 5 hr.	P-170 A-170				P-150 A-148			
O.L.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	P-10 A-10			P-9 A-10				P-10 A-10
A.R.	syphilis of c. n. s.	0.3 c.c. ++	++	clear	clear	P-7 A-7		P-7 A-6				P-6 A-5	
O.C.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	P-11 A-11			P-12 A-11				P-11 A-10
J.P.	gastric neurosis	2.0 c.c. –	–	clear	clear	P-1 A-0			P-2 A-2				P-1 A-1
J.O.	diabetes mellitus	2.0 c.c. –	–	clear	clear	P-0 A-1			P-2 A-1			P-1 A-0	

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOBULIN	MACROSCOPIC APPEARANCE		CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
				IMMEDIATELY	ON STANDING								
H.E.L.	syphilis (not of c. n. s.)	2.0 c.c. –	–	clear	clear	P-3 A-3			P-2 A-2				P-3 A-2
R.P.	encephalitis lethargica	2.0 c.c. +±	–	clear	pellicle in 5 hr.	P-53 A-50				P-90 A-63			A-58
R.S.	neurasthenia	2.0 c.c. –	–	clear	clear	P-6 A-4			P-5 A-4				P-4 A-3
F.B.	mitral stenosis	2.0 c.c. –	–	clear	clear	P-3 A-3				P-3 A-4			A-3
R.P.	encephalitis lethargica	2.0 c.c. –	+±	clear	pellicle in 1 hr.	A-53 P-54		A-62 P-90				A-58 P-65	
M.C.	syphilis (not of c. n. s.)	2.0 c.c. –	–	clear	clear	A-2 P-2				A-1 P-2		A-1 P-1	
L.H.	syphilis of c. n. s.	0.5 c.c. ++	++	clear	clear	A-7 P-9		A-8 P-8					A-7 P-8
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-5 P-7				A-6 P-7		A-6 P-6	
G.Sn.	tabes dorsalis	0.7 c.c. ++	++	clear	clear	A-11 P-13				A-12 P-14			A-12 P-16
E.L.	syphilis of c. n. s.	0.8 c.c. ++	+±	clear	clear	A-15 P-18			A-16 P-19		A-19 P-21		
J.N.	syphilis of c. n. s.	0.8 c.c. ++	++	clear	clear	A-14 P-15		A-14 P-13				A-12 P-12	
O.C.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	A-10 P-10						A-8 P-10	
M.Z.	syphilitic meningitis (treated)	0.4 c.c. ++	++	clear	clear	A-7 P-9	A-7 P-8					A-6 P-7	
R.B.	tabes dorsalis	0.4 c.c. ++	++	clear	clear	A-8 P-10			A-7 P-9			A-7 P-10	
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-9 P-9			A-7 P-9				A-8 P-8
J.W.	c. n. s. lues	0.4 c.c. ++	++	clear	cloudy in 3 hr.	A-30 P-32			A-16 P-19	A-16 P-18			A-11 P-12
O.C.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	A-12 P-15						A-11 P-12	

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOBULIN	MACROSCOPIC APPEARANCE IMMEDIATELY	MACROSCOPIC APPEARANCE ON STANDING	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
L.S.	neurosis	2.0 c.c. –	–	clear	clear	A-2			A-0			A-1	
						P-2			P-1			P-1	
L.H.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	A-10		A-11				A-11	
						P-12		P-12				P-13	
A.G.	psychoneurosis	2.0 c.c. –	–	clear	clear	A-5	A-4					A-4	
						P-5	P-5					P-4	
E.H.	luetic myelitis (treated)	0.6 c.c. ++	++	clear	clear	P-19						A-17	
						A-17						P-17	
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-15						A-14	
						P-16						P-14	
X.R.	acute anterior poliomyelitis	2.0 c.c. –	++	clear	sed. = 12 hr.	O-230	O-228		O-230			O-180	
						5-232	5-230		5-228			5-174	
						sp230	sp234		sp226			sp176	
S.L.	acute anterior poliomyelitis	2.0 c.c. –	+–	clear	central web = 3½ hr.	O-17	O-18	O-16	O-450				
						5-17	5-17	5-14	5-447				
						sp15	sp16	sp17	sp442				
R.B.	neurosis	2.0 c.c. –	–	clear	clear	P-1					P-3		
						A-1					A-1		
M.Z.	syphilitic meningitis (treated)	0.4 c.c. ++	++	clear	clear	P-16			P-20			P-16	
						5-18			5-20			5-17	
						sp16			sp19			sp16	
R.B.	tabes dorsalis	0.4 c.c. ++	++	clear	clear	P-63			P-61				P-58
						5-57			5-64				5-61
						sp55			sp62				sp57
O.C.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	P-22				P-19			P-21
						A-20				A-19			A-20
J.W.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	P-4		P-4		P-3			P-4
						A-4		A-3		A-4			A-4
H.S.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	P-17			P-15			P-17	
						A-16			A-16			A-15	
J.P.O.	tabes dorsalis	1.0 c.c. +±	++	clear	clear	P-25			P-27				P-27
						A-23			A-26				A-27
L.S.	syphilis (not of c. n. s.)	2.0 c.c. –	–	clear	clear	P-2		P-1					P-2
						A-1		A-1					A-1

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOBULIN	MACROSCOPIC APPEARANCE IMMEDIATELY	MACROSCOPIC APPEARANCE ON STANDING	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
F.D.	neurosis	2.0 c.c. –	–	clear	clear	P-3		P-2				P-3	
						A-3		A-3				A-2	
J.Q.	syphilis of c. n. s. (paresis)	0.2 c.c. ++	++	clear	hazy at 5 hr.	P-78				P-77		P-85	
						A-76				A-75		A-84	
C.B.	encephalitis lethargica	2.0 c.c. –	–	clear	clear	A-30			A-27				A-27
						P-30			P-31				P-25
R.B.	tabes dorsalis	0.3 c.c. ++	++	clear	clear	P-60					P-61		
						A-60					A-58		
K.L.	acute anterior poliomyelitis	0.2 c.c. –	++	clear	sed. 2 hr. cen. web 12 hr.	P-140	P-144	P-118	P-120			P-41	
						5-130	5-135	5-110	5-114			5-36	
						sp130	sp130	sp112	sp115			sp38	
G.N.	syphilis of c. n. s.	0.5 c.c. ++	++	clear	clear	P-10	P-10	P-14	P-15				P-10
						5-11	5-11	5-15	5-10				5-12
						sp11	sp10	sp12	sp11				sp10
E.Q.	lateral sclerosis	1.0 c.c. ++	++	clear	clear	P-3	P-5	P-5					P-6
						5-4	5-5	5-4					5-4
						sp3	sp4	sp4					sp5
C.C.	?acute anter. poliomyelitis	2.0 c.c. –	+–	clear	clear	P-22			P-18		P-21		
						5-23			5-22		5-21		
						sp21			sp23		sp17		
M.M.	acute anterior poliomyelitis	2.0 c.c. –	++	clear	central web in 15 hr.	P-232	P-238	P-240				P-80	
						5-236	5-238	5-236				5-78	
						sp238	sp242	sp240				sp76	
M.Z.	encephalitis lethargica	2.0 c.c. –	+–	clear	clear	P-2			P-2				P-2
						A-2			A-1				A-2
F.V.	encephalitis lethargica	0.5 c.c. ++	–	clear	clear	P-27		P-26					P-26
						A-26		A-25					A-25
G.L.	tabes dorsalis	0.5 c.c. ++	++	clear	clear	A-6			A-6				A-5
						P-6			P-8				P-6
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-16				A-15			A-16
						P-15							
L.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	P-14			A-16				A-14
						A-14							
A.F.	aortic insufficiency	2.0 c.c. –	–	clear	clear	P-2					A-2		A-3
						A-2							

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOBULIN	MACROSCOPIC APPEARANCE		CELL COUNT	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
				IMMEDIATELY	ON STANDING	0 HR							
S.S.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	P-32 A-30				A-32			A-32
H.D.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	P-7 A-7			A-7				A-6
H.W.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	P-11 A-10				A-9			A-9
E.R.	tabes dorsalis	0.5 c.c. ++	++	clear	clear	P-15 A-14				P-14 A-14			A-13
P.R.	syphilis (not of c. n. s.)	2.0 c.c. –	–	clear	clear	P-1 A-1			A-1				A-1
C.T.	syphilis (not of c. n. s.)	2.0 c.c. –	–	clear	clear	P-1 A-0				P-1			A-1
A.F.	syphilis of c. n. s.	0.5 c.c. ++	++	clear	clear	P-50 A-48		P-49 A-48				P-49 A-50	
H.W.	syphilis of c. n. s.	0.5 c.c. ++	++	clear	clear	A-6 P-5			A-5 P-6				A-6 P-6
J.N.	syphilis of c. n. s.	0.8 c.c. ++	++	clear	clear	A-69 P-69				A-69 P-68			A-68 P-68
W.S.	tabes dorsalis	0.8 c.c. ++	++	clear	clear	A-5 P-5			A-4 P-4				A-5 P-4
B.B.	chronic diarrhea	2.0 c.c. –	–	clear	clear	P-1 A-1				P-0 A-0			P-1 A-0
H.D.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	P-5 A-4			P-4 A-4				A-3 P-4
W.S.	tabes dorsalis	0.8 c.c. ++	++	clear	clear	P-10 A-8				P-10 A-9			P-9 A-9
C.N.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	P-25 A-23		P-25 A-24				P-24 A-24	
A.B.	syphilis of c. n. s.	0.5 c.c. ++	++	clear	clear	P-5 A-5				P-4 A-3		P-5 A-5	
J.N.	syphilis of c. n. s.	0.8 c.c. ++	++	clear	clear	P-18 A-16					P-16 A-14		P-17 A-17
W.S.	tabes dorsalis	0.8 c.c. ++	++	clear	clear	P-13 A-11				P-11 A-10			P-12 A-11

cases subsequently on the wards over a period of many months. The results of counting after such a procedure in these two cases are shown in Table II.

TABLE II

GROSS APPEARANCE—TYPE IV PNEUMOCOCCUS MENINGITIS			
Tube plain fluid		Tube citrated fluid	
immediately opalescent	on standing central funnel web in one hour	immediately opalescent	on standing no change in 15 hours
CELL COUNTS			
	at 0 hr	at 3 hrs.	at 15 hrs.
plain tube	3000	impossible in one hr. because of web	not possible
citrated tube	3000	2990	2994
GROSS APPEARANCE—SYPHILITIC MENINGITIS			
Tube plain fluid		Tube citrated fluid	
immediately opalescent	on standing unchanged in 15 hr.	immediately opalescent	on standing no change in 15 hr.
CELL COUNTS			
	at 0 hr	at 3 hrs.	at 15 hrs.
plain tube	2500	2510	2489
citrated tube	2504	2490	2496

Note:—All counts made with undiluted fluids.

The following conclusions seem warranted from the foregoing data:

(1) The cells in clear spinal fluids, collected in clean tubes and tightly stoppered, may in the absence of macroscopic pellicle, sediment, or web be safely counted if thoroughly mixed at any time up to at least fifteen hours. If the spinal fluid is not clear or becomes clouded, the results of counts at different times are apt to vary.

(2) It is desirable but not necessary for fluids to be collected in sterile tubes.

(3) Whether the fluids are kept at room temperature or in the ice box seems to be of little consequence.

(4) In two cases of meningitis, the thorough mixture of small quantities (0.2-0.5 gm. for 6 c.c.) of powdered sodium citrate with the fluids made it possible to duplicate the original cell counts (within the limit of technical error) at three and fifteen hour intervals. The procedure must be tried in a large series of cases before any conclusions are warranted regarding such citrating of fluids.

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ON THE DETERMINATION OF SMALL QUANTITIES OF ATROPINE IN BLOOD SERUM*

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IN PREPARING a paper on the natural immunity of the rabbit for atropine which has appeared in an abbreviated form in the Arch. Néerl. de Physiologie (T V, 3 me Livr., p. 380, 1921), I had to examine all alkaloid reagents with regard to their applicability for the quantitative determination of atropine, because I found that no one had done this before me. As I think that this preliminary work, which cost me much time, may be of some use to later students of this subject, I publish herewith its results.

The determination of small quantities of the alkaloid in blood serum can immediately be divided into two parts: (1) The isolation of the alkaloid out of the complicated chemical mixture which we call serum. (2) The determination of atropine in the watery solution thus obtained. I shall treat these two parts separately.

1. Isolation of the alkaloid

In forensic chemistry such a case would demand the method of Stass-Otto, which has been accurately described in the *Lehrbuch der gerichtlichen*

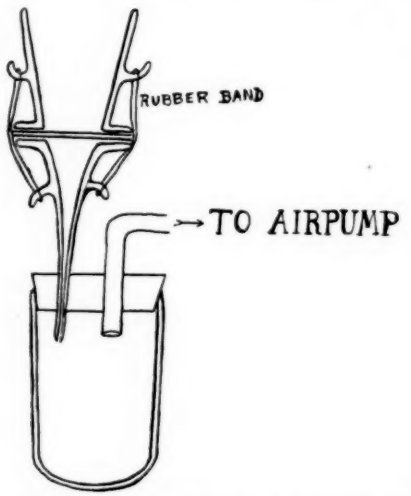

Fig. 1.—Apparatus for microfiltration. The liquid passes through two pieces of filter paper kept tightly between the two pieces of the apparatus by means of rubber bands.

Medizin of Baumert. Without comparing it in detail with the other existing methods, as for instance that of Kippenberger—the bibliography showed me that one of them is as good and bad as the other—I immediately tried to

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adapt this method to microchemical analysis. Using small test tubes, containing about 15 c.c., with a thick wall, so that they could be used in the centrifuge, the amount of atropine was determined in one c.c. of serum, containing various amounts of atropine dissolved in one c.c. of water. Immediately after they had been taken out of the incubator, they were filled with absolute alcohol, and thoroughly shaken. The precipitate was collected on the bottom by means of a strong centrifuge, and the completely clear supernatant liquid decantated. Then the proteins were washed out several times with absolute alcohol, and all the alcohol united in a beaker. As I usually had a number of determinations to do at the same time, I boiled them all down on a large sand-bath. In this case one must be very careful to prevent bumping and to take them off at the very moment that they get dry, otherwise the material is roasted, and the determination spoiled.

The residue is now taken up in water and boiled down to a few c.c. Droplets of fats appear as soon as the preparation is allowed to cool. The liquid, in which a small precipitate sometimes has been formed, principally consisting of fat, is now filtered off by means of a microfilter (Fig. 1). The volume of the watery atropine-solution now obtained—which is completely clear and contains moreover perhaps some inorganic salts and some amino-acids in negligible quantities—was determined by weighing.*

2. DETERMINATION OF THE QUANTITY OF ATROPINE IN THE SOLUTION

The methods of determination of atropine can be divided into five groups. They are: A. Physiological methods. B. Color reactions. C. Precipitate reactions. D. Titrimetrical methods. E. Capillary-analytical methods.

A. Of these I know five which could be used for our purpose. A very sensitive method has been devised by Fleischmann,[1] by which even 10^{-8} gm. can be detected. In the frog heart, which has been inhibited by means of muscarine, he injected by means of a Pravaz-syringe the mixture of serum and atropine, in which the quantity had to be determined. This method can, however, not be used for our purpose because the quantities of atropine and muscarine, which compensate each other, are different in different cases. However accurate this method may be it can only be used to detect very small quantities qualitatively. The second method is that of Metzner,[2] who used the stimulation of the N. vagus for this purpose. This method is as well as that of measuring of the mydriasis, the third one, not sensitive enough for our purpose. In the fourth place I wish to call the attention to the work of Storm van Leeuwen,[3] who working in this line used the movements of a surviving gut for the estimation of the alkaloid, according to the method of Magnus. This method as well as that of Cushny[4] who uses a dog with a salivary fistula, can only be used for a few experiments; when a whole series of estimations has to be made, both take too much time and material. Moreover these methods have always the disadvantage of depending on many other factors (tem-

*In order to be sure, that in this way all the atropine was obtained, I made a control experiment in the following way. The precipitates of about ten determinations were collected, boiled and extracted with about all atropine-solvent reagents, such as alcohol, ether, water and hydrochloric-alcohol. The whole mass was then neutralized, boiled down in the same way as above described, and taken up in a few drops of water. No atropine could be demonstrated herein.

perature, condition of the animal, etc.), so that for an accurate quantitative determination we can better use a chemical method.

B. There are a great number of qualitative color reactions for alkaloids. For quantitative purposes with reference to atropine, none of them can be used as the following review will show.

1. *The reaction of Vitali.*—This is the most well known of the color reactions for atropine.[5] The solution is evaporated on a water-bath with concentrated nitric acid. After it has been allowed to cool, alcoholic potassium hydroxide is added. The solution gets a red color, if atropine is present. There is however no possibility to adapt this method to quantitative purposes—for instance with a color scale or with a colorimeter, because as I could state several times, the color changes every moment. In accepting the results which several authors obtained in using this reaction, one should for this reason be very careful.

2. *The reagents of Wasicky.*[6]—A. With perhydrol-sulphuric-acid the three alkaloids, atropine, hyoscyamine and scopolamine assume a color, which is first leaf-green, then olive-green and finally brown-green. For the same reason as mentioned for the reaction of Vitali, this method cannot be adapted for quantitative use. That atropine should assume a color, when treated with perhydrol-sulphuric-acid, is, however, denied by Springer (Pharm. Ztg., 1902, p. 157), neither could I see any coloration. Probably the color seen by Wasicky is due to impurities. B. The reaction with p-dimethylbenzaldehyde—a cherry-red color—is still more sensitive. In how far this reaction can be adapted for quantitative use, must still be investigated. The author ascertains that the color remains constant for days. As it is practically impossible to obtain this reagent in sufficient quantities, we may safely exclude it for laboratory-practice.

3. *Sulphuric acid,* mentioned by several authors (recently Tunmann[7]) as a color reagent to alkaloids in general and to atropine particularly, gives, as already stated by Eder,[8] no color with atropine, not even when it is mixed with H_2O_2 (*perhydrol-sulphuric-acid*), with ammonium- or alkali-persulphate (*the reagent of Caro*), with $KMnO_4$ (1 gm. in 200 c.c. H_2SO_4) (*the reagent of Wenzel*), with HNO_3 (*the reagent of Erdman*), with a trace of iron (*the reagent of Keller*), with a watery solution of permanganate (*the reagent of Flückinger*), or with benzaldehyde (in alcoholic solution 1:5) (*the reagent of Melzer*).

4. *Nitric acid,* mentioned several times as a general alkaloid-reagent, gives no coloration with atropine.

5. *The reagent of Schlagdenhauffen,* a pyrogallol-solution, gives no color with atropine, neither when the solution is warmed.

6. *The reagent of Selmi,* a saturated solution of iodic acid, gives with some alkaloids a color reaction, due to the reduction of iodic acid.* Atropine, however, seemingly does not give this reaction.

7. *The reagent of Watson-Smith,* $SbCl_3$, colors many alkaloids. Atropine is not mentioned among them. Moreover this reagent is rather dangerous, as SbH_3 is easily formed.

*Stein[9] worked out a colorimetric method based upon this reaction.

8. *The reagent of Fraude,* perchloric acid with a specific weight of 1.13-1.14 cannot be used for atropine. The author used it especially for aspidospermine and the Strychnos-alkaloids. According to Häussermann and Sigel (Chem. Ztg., 1901, p. 32) pure perchloric acid (prepared from Ag-perchlorate and H_2SO_4) does not give this reaction. It is probably due to traces of Cl or ClO_2.

9. *The reagent of Lenz,* KOH, gives no color with atropine.

10. *The reagent of Archetti* (Ztschr. f. anal. Chem., 1901), a solution of potassium ferricyanide in HNO_3, gives an olive-brown color with atropine. This reagent has been used for quantitative purposes by Kieffer, but seems not to be very reliable.

11. *The reagent of Lucchini* (Chem. Kal., II, 695), a solution of potassium bichromate in concentrated H_2SO_4 gives no color with atropine.

12. The following reagents have been mentioned in the literature as untrustworthy, some of them have been excluded for practical reasons: *Urbansky and Mylius* (5 drops of furfurol in 10 c.c. H_2SO_4), *Frödhe or Buckingham* (1 gr. $(NH_4)_2MoO_4$ in 100 c.c. H_2SO_4), *Mandelin* (1 gm. ammonium-vanadium-sulphate in 200 c.c. H_2SO_4), *Sonnenschein* (ceriumsesquioxide in H_2SO_4), *Lafon or Ferreira de Silva* (ammonium selenite in conc. H_2SO_4), and the reaction of *Schneider,* using H_2SO_4 and sugar, the color being due to the formation of furfurol.

13. CN-gas colors a concentrated alcoholic atropine solution reddish-brown.[10] As our purpose is to determine small quantities, we can exclude this reaction.

Considering our results with the color reactions of atropine, we may safely conclude, that none of them can be used for quantitative purposes with enough accuracy. Part of them give no color at all with atropine, part of them give a color, which is too unstable to be used colorimetrically.

C. Let us now consider the next group, that of the precipitate reactions. These are also known in great number.

1. *The reaction of Ladenburg.*[11] $AuCl_3$ (1:20) gives a faint yellow precipitate in a solution of atropine. When looked at under the microscope, it appears to consist of oily drops. Left to itself, it crystallizes overnight in beautiful small rosettes. Addition of a trace of hydrochloric acid (not too much) accelerates the crystallization. The crystals can now easily be centrifuged into a calibrated capillary tube (as they have first been described by Hamburger in the Biochemische Zeitschrift, 1906, i, 263) and measured. As this method seemed to promise beautiful results, I studied it very carefully, but after some time I saw that it could not be trusted quantitatively, because the salt is still too soluble in water. Until a dilution of about 1:200 was reached, the results were rather satisfactory. One part of the scale appeared to indicate 0.0198 mgm. alkaloid, so that the test is rather sensitive. But in greater dilution—and I always had to determine the amount of atropine in more dilute solutions—it was not to be trusted, and gave very irregular results because of the solubility of the gold-salt. For this reason I gave up this nice method, although I think, that it would be worth while to try to find out means to use it.

2. *The reaction of Duflos.*[12] $PtCl_4$ gives an analogous precipitate.

3. *The reagent of Mayer*[13] *of Valser,* $KI.HgI_2$, and

4. *The reagent of Dragendorff,*[14] $KI.BiI_3$, give gray or brown precipitate with all alkaloids. The sensitiveness of the reaction is greater in acid solution than in a neutral one. The precipitate cannot be determined in the way in which I determined the gold-precipitate, because it adheres to the walls of the vessel, and is not collected at the end of the capillary, neither is it possible to determine indirectly the amount of atropine in determining the mercury, because the precipitate is not constant enough in composition. This was already stated in 1898 by A. B. Prescott,[15] where he says: "The alkaloidal bismuth iodides are not quantitatively uniform enough for alkaloidal assay; but are more stable and less uniform than the alkaloidal mercuric iodides formed by Mayer's reagent. * * * I am well aware how unsatisfactory the latter has been found in the hand of analysts."

There is, however, one way of using this reagent, which I will describe, and which gives rather reliable results. Though this method is still far from being an ideal one, I used it, because a better one could not be found among the 60 or more reagents which I studied for this purpose. My method consists in dividing a part of the watery atropine solution, which I had obtained, as described in the first chapter, into a number of equal parts. When only small quantities of liquid are available, one can simply bring one drop in each of a series of watch-glasses. These equal parts are then diluted with one volume of water, two volumes and so on. Mayer's reagent is now added in equal quantities, and the dilution determined in which the reagent still gives a precipitate. The determination of this limit is very delicate, and it requires some exercise to get the necessary routine. Moreover, the limit is not the same under different conditions of light and for different observers. In my work on the natural immunity of the rabbit for atropine I made for this reason in each series of experiments two control experiments. As the only important information for a physiologist consists in relative data, and the absolute data are mostly rather indifferent, this error is of no great importance. When enough of the solution is available, one can still make an interpolation between the dilution at which he just sees a precipitate, and the next one, though I would not advise this as it lies beyond the limit of accuracy which we can attribute to this method.

An important thing to keep in mind is the fact that according to Kunz[16] the limit is influenced by alcohol, acetic acid, ammonia, glycerine and proteins. It is therefore of the utmost importance, that one be quite sure that the alcohol is quite evaporated, before he takes up the residue in water (see Chapter I), and that one uses fresh sera, in order to avoid the presence of ammonia to any considerable extent. Moreover it is advisable to use exactly the same serum in the control determinations and the experiment. In spite of all these difficulties, I took this method out of the 60 or more mentioned in this paper, because it is the only one which gives approximately reliable results, and is the least objectionable of all.

5. Analogous salts of Ba,Ca,Sr,Cd,Zn, etc. ($KI.CdI_2$, *Marmé's reagent*),

may be used as well as the Hg and Bi salts. An investigation of Herder,[17] however, showed that the sensitiveness increases with the molecular weight of the metal, so that there is no reason to prefer them.

6. *The reagent of Bouchardat,* I in KI, can be used titrimetrically. For this I refer to the next part.

7. The same can be said of *the reagent of Grandeau,* Br in BrK.

8. *The reagent of Hager,*[18] picric acid, is not trustworthy as a reagent as especially the critic of van den Burgh[19] has shown. Though Schaer[20] demonstrated that of the 18 nitro-compounds, which he studied as alkaloid-precipitants, picric acid gave the most reliable results, even these results are not so very satisfactory. Only in etheric solution the picrate precipitates quantitatively, according to Chandelon.[21] In the *Compt. Rendus* 123,929, E. Pozzi-Escot demonstrates, moreover, that picric acid cannot even be used for qualitative analysis.

9. *The reagent of Knorr* (picrolonsäure, i.e., dinitrophenylmethylpyrazolon) cannot be trusted for quantitative work. For the literature see reference 19.

10. Phosphomolybdic acid (*de Vry and Sonnenschein's reagent*), phosphotungstic acid (*Scheibler's reagent*) and phosphoantimonic acid (*Schultze's reagent*), used in their Na salts, might be used in the same way as Mayer's and Dragendorff's reagents. As however the limit of their sensitiveness is lower, our reagent is to be preferred.

11. *Tannic acid* precipitates not only alkaloids, but also proteins, so that it is safer not to use it. Moreover, the results obtained with this reagent are not so very sharp.

12. *The reaction of Veltmann*[22] which has been used in numerous modifications, is based on the precipitation of the atropine with ammonia. As, however, this reaction is not complete, it is better not to use it.

13. The following reactions have been excluded partly because they were mentioned in the literature as absolutely untrustworthy, partly because they do not give any reaction with atropine at all and partly because it is too difficult to obtain them: potassium platinum cyanate; potassium platinum cyanide; metatungstic acid; sodiumnitroferrocyanide (Na_2FeCy_5NO); potassium cuprocyanide; $PbCl_2$; aqueous solutions of Br and Cl; $K_2Cr_2O_7$; potassium ($Pt(SCy)_4.2KSCy$), the so-called salt of *Buckton* (in 4 per cent sol.); KSCy; $FeCl_3$; silico-tungstic acid; Ir-chloride; Pd-chloride; Na-sulphoarseniate; Na-sulphoantimoniate (the so-called salt of Schlippe); boric-tungstic acid; seleno-sulphuric acid and formaline-sulphuric acid.

D. 1. The *alkalimetric determination* method is as old as the determination problem itself. Schloessing in the Arch. d. Pharm., 1847, ci, 63, was the first to work out a method in this direction. On the question what indicator has to be used opinions are still divergent. I refer to a paper on this subject of E. Runne in the Apoth. Ztg., Berlin, 1909, xxiv, 662. This method is not advisable for our purpose because of small quantities amino acids, carbonates, fats and perhaps phosphates, which may be expected in the liquid. Good methods are those of Elie Falieres[23] and of Elias Elvove.[24]

*Schultze's reagent gives no reaction with atropine according to the Chem. Kal.

2. A second titrimetric method is the *iodometric method.* Rudolf Wagner, Dingler's Journal, 1861, clxi, 40, was the first to use it. A very good and reliable method has been worked out by Gordon and Prescott[25] which is based on the formation of atropine enneaiodide in very dilute solution. In some previous experiments I could state, that it can be very well adapted to microchemical use; the presence of traces of proteins and fats which absorb the iodine prevents us from using this method for our purpose.

3. Mayer's reagent and that of Dragendorff are sometimes used titrimetrically. Because of the small quantities of atropine our method is, however, to be preferred.

4. For morphine: Its reducing qualities have been used by Kieffer[26] and Reichard.[27]

E. Our last method of determination of alkaloids is a very sensitive one, the capillary-analytical method, in which the stalagmometer of Traube is used. It is, however, so very sensitive, that the smallest quantities of inorganic salts disturb the determination. For this reason it cannot be used for our purposes.[28]

Exclusively qualitative are two odor-reactions on atropine.[29] (Cited after 9.)

1. When atropine is heated with conc. H_2SO_4, it smells like orange-blossom.

2. Heated with conc. H_2SO_4 and some crystals of ammonium molybdate, it smells like bitter almonds.

SUMMARY

Atropine has to be determined quantitatively in 1 c.c. of serum, containing maximally 5 mgm. alkaloid. In test-tubes of about 15 c.c. the proteins are precipitated with absolute alcohol. The precipitate is washed out several times with alcohol, and does not contain such a quantity of alkaloid as can be demonstrated. The alcoholic solution is evaporated. The residue is taken up in water. This watery solution is concentrated to a few c.c. A precipitate principally consisting of fats is filtered off with a microfilter. The quantity of atropine in this watery solution is determined with the reagent of Mayer, $KI.HgI_2$, which appeared to be the least objectionable of 60 or more tested reagents. The dilution is determined in which this reagent is just capable to give a precipitate. From this the quantity of atropine can easily be calculated. Some sources of error are indicated on p. 284. Besides the experiment one should always make a control determination!

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THE PHENOMENON OF BACTERIOPHAGE*

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BACTERIOPHAGE is a recently recognized phenomenon which is due to the activity of a substance occurring under certain conditions in bacterial cultures which has the property of destroying bacteria. The peculiar properties of this principle have stimulated considerable research in regard to its nature and the possible relation it may have to immunity. The discovery of Bacteriophage or d'Herelle's phenomenon, as it is frequently called, has recently been attributed by Doctor Jules Bordet[1] to F. W. Twort[2] who published an article in *The Lancet*, London, in 1915, describing in detail the process of autolysis which he observed in agar colonies of a micrococcus developing from calf vaccinia. Since 1917 detailed studies of this phenomenon have been made by d'Herelle, by Kabeshima and by Bordet and Ciuca. There have also been contributions by Gratia, Salimbeni, Kuttner, Maisin, Dumas, Elvira and Pozerski, Wollman, Debre and Haguenau, and Bablet.

The conflicting theories to explain this interesting phenomenon are best understood if preceded by a brief description of the facts available. An active lytic substance such as d'Herelle employs[3] is obtained from the feces or urine of a patient suffering from dysentery. An emulsion is made in broth and the material filtered. Bordet and Ciuca,[4] by inoculating a guinea pig intraperitoneally with several doses of B. Coli obtained a peritoneal exudate which had this same property of dissolving or inhibiting a culture. This lytic principle was at first thought to be specific for the causative organism but it is now known to be active against a number of different strains and even under certain conditions against a number of related groups of organisms. This lytic property can be transmitted in series indefinitely either from very minute quantities of an inhibited or dissolved culture or from an agar colony which is resistant to the action of the lytic substance. This activity is only demonstrable in young living cultures.

In the first investigations carried out by Twort[2] the phenomenon was observed as a slow lysis of bacterial colonies developing on agar from glycerinated calf vaccinia. It was also found to have some activity on staphylococcus albus and aureus, but not on streptococcus or B. Coli. He discussed the possibilities of several theories to explain this lytic activity, namely: (1) The presence of an ameba form; (2) a stage in the life history of the micrococcus which will not grow in ordinary media; (3) a minute bacterium; (4) a still lower form of life (protoplasm without form or an enzyme with the power of growth); (5) an enzyme secreted by the micrococcus. He was inclined to

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believe it is such an enzyme or, he states, it "might rather be accepted as an acute infectious disease of the micrococci." He demonstrated the activity of this substance after passing it through the finest filter and after heating at 52° C. for one hour. He found it maintained its activity for six months on agar but that it would not itself grow on any medium. It was not of an infectious nature.

F. d'Herelle[5] has maintained from the first that the bacteriophage activity is due to a diastatic action of a resistant filterable organism—a bacterium, parasite or protozoan. He has worked principally with B. dysenteriae Shiga, using filtrates of a broth suspension of the excreta of dysentery patients. He considers[6] the lytic substance a normal host of the intestines, during health adapted to the destruction of B. Coli but in dysentery adapting itself to B. dysenteriae. The principle is obtained in any intestinal disease as primarily specific for the causative organism,[7] the activity depending on the virulence of the bacteriophage organism rather than the numbers present. D'Herelle describes an abnormal form in a culture in the process of dissolving[8] which is visible by the use of the ultramicroscope. It is a spherical form supposedly of the organism containing the multiplying bacteriophagic organism. It is seen rupturing and scattering many minute bodies into the medium. This liberates the first generation of the parasitic organism along with a diastase. Ordinary microscopic examination can reveal nothing because—1st, an active filtrate can pass through a collodion membrane which limits molecules of serum albumin, and 2nd, a beam of light does not detect particles in such a solution. He states, however, that there is a sediment on centrifugalization or on standing. The action of physical and chemical agents such as heat, time, ether, glycerine he believes to be compatible with life and cites analogous experiments with organisms. In explaining the action of this bacteriophagic organism, d'Herelle indicates[9] the possibility that a bacillus may acquire an immunity against an infection of such a parasite, and thus would be explained the nature of relapses in typhoid. Immunization, he says,[8] has two phases, the active lysis, and an indirect action that modifies the bacterium. A protection against infection has been demonstrated by the use of a dissolved culture, and in human infections of dysentery five cases were cleared in 24 hours by the ingestion of such a substance.[10] D'Herelle also finds that an antilytic serum is produced by the introduction of bacteriophagic culture filtrates in the animal body. This, he contends,[11] does *not* destroy the bacteriophagic organisms, but merely inhibits them for a length of time depending on the amount of serum used. This antibacteriophage serum also has a powerful antiimmunizing power.

Bordet and Ciuca[4] obtain the lytic principle from a filtrate of a peritoneal exudate produced by inoculating a guinea pig repeatedly with B. Coli. They believe it is a phenomenon of defense due to the leucocytes which give to the organism an hereditary property of producing a lytic ferment. This ferment is capable of being diffused in a liquid medium and affecting normal organisms of the same species. Subsequent generations of a lytic strain inherit this new property. The acquired characteristic[12] may be due to a temporary

external factor which breaks the equilibrium between the construction and destruction of living matter. This variation operating for the first time on a bacterium is transmitted by it to its descendants. It is also diffusible in culture media and will cause a new modified race by merely coming in contact with normal bacteria. Therefore the variation may be considered not only hereditary but contagious. Bordet and Ciuca[13] also obtained an antilytic serum by immunizing with a dissolved culture or with a strain of B. coli obtained from an organism developing in a culture which partially resists the action of a lytic fluid. This antilytic serum they find to inhibit the action of ten times the amount of lytic fluid—in other words, they believe there is a direct neutralization of this substance. The explanation of the persistence of B. coli in the intestines in the presence of such a lytic substance is suggested by an experiment in which invulnerable organisms are produced, that is, a strain having no power to lyse a normal culture and not being dissolved itself by a lytic fluid. This organism is produced by the action of a lytic substance on a normal culture of B. coli over an extended period of time.

Recent work by André Gratia has been especially directed towards the study of the behavior of organisms resistant to the action of the lytic fluid, in a comparison with sensitive strains. Using a culture of normal B. coli he has found[15] that after a culture is partially dissolved the resistant organisms are characterized by (1) enhanced virulence; (2) greater motility; (3) slow growth in broth with a late pellicle formation and a sediment which is not compact, but with a clear supernatant fluid; (4) a diffuse growth in an agar stab; (5) characteristic transparent irregular colonies on agar; (6) in peritoneal infections it produces an exudate of a serous nature. Gratia has also found[16] that a normal culture of B. coli which is allowed to become old and dry contains certain organisms which are resistant to the lytic substance. He believes that ageing and the lytic principle both have a selective action rather than an action founded on hereditary adaptation. Gratia has found[17] that the most favorable reaction of a medium for the production of a lytic substance is slightly alkaline, and he has also worked on the specificity of the principle, concluding that by successive passages with allied species of organisms the bacteriophage becomes acclimated and will act as a specific lytic agent. He finds[18] a lytic fluid obtained from a resistant culture of B. coli is not as specific as one obtained from a very sensitive culture.

Among other theories of this phenomenon we have that of Kabéshima.[19] He believes the activity of such a lytic substance is due to a "ferment d'immunité bactériolysant" or that there occurs in the glands of the intestines a catalyzer which acts on a prodiastase in the microorganism forming a ferment which is a catalyzer for organisms of a new generation. He especially refutes d'Herelle's theory by showing that the properties of the lytic substance[20] are similar to those of an enzyme rather than of a living organism. For instance he precipitates the active principle with acetone and extracts it with ether. D'Herelle[21] points out that B. subtilis may be precipitated by the same method and even after drying resistant forms will persist and develop in broth. He does not find the principle soluble in ether. Kabéshima found as did d'Herelle

that a sediment occurs on centrifugalization of an active filtrate but this sediment he found no more active than the supernatant fluid. D'Herelle finds the greatest resistance to heat exhibited by the lytic principle after it has stood about fifteen days. Heating at 68° C. for one hour immediately after the lysis of a culture is complete, diminishes the lytic power considerably. Thus he shows the similarity to resistant forms of microorganisms. By counting the number of clear zones appearing on agar growths with the addition of a bacteriophage substance d'Herelle computes the number of active individuals per c.c. Then using a very small amount of this highly diluted in ten tubes of a B. dysenteriae Shiga culture he found lysis occurred in three of the tubes. This he took as an indication[22] that there were present active individuals rather than a diffusible enzyme.

Salimbeni claims to have found a myxameba in lytic filtrates which he has named "myxomyces shigaphagus."[23]

J. Dumas[24] has reported the presence of a bacteriophagic substance active against B. coli and B. dysenteriae Shiga, but not against cholera, in normal feces, soil, city water, Seine river water and conduits. He finds the presence of broth is necessary to produce a lytic fluid. In some relation to this last fact, perhaps, Miss Kuttner[25] notes that in a cleared culture if the tube is reclouded, fresh broth must be added before more of the lytic principle will be active.

As can be seen from this brief survey of the literature, the interest in this phenomenon lies principally in solving the problem of the nature of the active principle and its function in infection and immunity. So far the problem has only been broached and its ultimate significance, although undoubtedly considerable, lies in the future.

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LABORATORY METHODS

GLUCOSE TOLERANCE TEST*

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DEFINITION

THE term "glucose tolerance test," or better, "blood sugar tolerance test," is applied to the series of blood sugar determinations following the ingestion of one hundred grams of glucose, showing what is known as the sugar tolerance curve.

In the average normal individual during the period of nonabsorption from the gastrointestinal tract the amount of dextrose in the blood remains constant between the limits of sixty and one hundred twenty milligrams per 100 c.c. of blood. During the period of absorption this amount is considerably increased—the postprandial hyperglycemia. Following the ingestion of one hundred grams of glucose the concentration of sugar in the blood begins to increase, reaching a maximum of not to exceed one hundred sixty milligrams at the end of forty-five minutes to one hour, and soon begins to decline rapidly, reaching approximately the original concentration by the end of two hours. This is known as the normal curve. (Solid line, Fig. 1.)

Macleod[1] states that during the period of nonabsorption the concentration of sugar in the portal vein is approximately the same as that in the systemic circulation, but during the period of absorption that of the portal circulation is considerably higher. The liver, interposed as a barrier between the two, has been proved capable of elaborating and storing glycogen to the amount of one hundred fifty grams; the muscles also are able to remove from the circulating blood and store an equal amount. The glycogen thus stored in the muscles constitutes the immediate supply of carbohydrate for body oxidation, that in the liver the reserve supply. But the liver and muscles are not able to care for all the absorbed sugar immediately, but allow some to pass into the general circulation, hence the temporary hyperglycemia following the ingestion of carbohydrates. The determination of the height and persistence of this postprandial hyperglycemia constitutes the tolerance test.

CONTROL OF CARBOHYDRATE METABOLISM

At every muscular contraction glucose is burned up and the reserve supply in the liver is drawn upon by a process of hydrolysis, through the action of an enzyme, glycogenase, either in the liver cells or in the circulating blood and lymph, the process being known as glycogenolysis. The mechanism

*From the Clinical Laboratories of the State University School of Medicine. Read before the Oklahoma County Medical Society April 9, 1921.

of this process is under control of the endocrine secretions, and is so finely adjusted that excessive concentration in the blood does not occur.

This adjustment is brought about to a limited extent by nervous control, as is evidenced by the so-called nervous glycosurias, the experimental glycosurias caused by stimulation of the splanchnic nerves, and the glycosuria following injury to the floor of the fourth ventricle. This control, however, is probably only secondary, due to hyperactivity of the suprarenal glands, from stimulation through the sympathetic system (McKenzie[2]). But for the most part the mechanism is under control of the internal secretions direct.

With reference to the process of glycogenolysis the internal secretions have been classified as (a) Inhibitors of glycogenolysis, and (b) Accelerators of glycogenolysis (Tidy[3]). Because of the accurate equilibrium of these two opposing forces, the sugar content of the blood is maintained at a constant level; if either becomes deranged the equilibrium is lost, and a hyperglycemia or a hypoglycemia results.

The secretion of the suprarenals is the most important of the accelerators of glycogenolysis. The hyperglycemia of depancreatized animals rapidly drops after removal of the suprarenals (McKenzie[2]); the injection of adrenalin will cause a hyperglycemia and glycosuria; in fact, Rhodenberg, Bernhard and Krebiel found the tolerance test can be carried out equally as well by the injection of one cubic centimeter of a $\frac{1}{1000}$ adrenalin solution as with the ingestion of one hundred grams of glucose. So in hyperactivity of this gland one will find a high blood sugar curve indicating a low carbohydrate tolerance. On the contrary a low sugar concentration and high tolerance is found in Addison's disease. The thyroid plays an important part as an accelerator of glycogenolysis, consequently in hyperthyroidism there is a hyperglycemia, while in cretinism and myxedema the opposite is true. The pituitary body is also an accelerator, and hyperglycemia and glycosuria are more or less constant in the early stages of acromegaly. These secretions probably inhibit the process of glycogenesis as well as accelerate the process of glycogenolysis.

On the other hand the internal secretion of the pancreas plays a most important part as an inhibitor of glycogenolysis, and is the main factor in the process of glycogenesis: and it is in deficiencies of this secretion that we find the most marked and most typical hyperglycemia and glycosuria, that of diabetes mellitus. The parathyroids play but a minor part as an inhibitor of glycogenolysis.

CARCINOMA AND CARBOHYDRATE METABOLISM

In addition to the endocrine disturbances mentioned there are a number of other conditions that disturb carbohydrate metabolism, notably tuberculosis and carcinoma, which appear to act very much as the accelerators of glycogenolysis.

Friedenwald and Grove[5] in summarizing the work on blood sugar determinations call attention to the statements of Freund and of Trinkler that there is always a hyperglycemia in carcinoma, a statement which we have not been able to substantiate. Many observers have noted the low tolerance to dextrose in these patients, and Rhodenberg, Bernhard and Krebiel[4] in 1919 and

1920[6] made extensive observations on nearly three hundred cases. In their preliminary report they described a very definite curve which they called the carcinoma curve. This occurred in twenty-four cases of carcinoma and one of sarcoma, and did not occur in forty cases of other diseases; in their second report[6] they found the same curve in certain other conditions, and some cases of proved carcinoma did not give the typical curve.

Friedenwald and Grove[5] reported studies in a series of thirty-two cases of gastrointestinal carcinoma in which they observed what they believe to be a rather characteristic curve, and which was not found in any of fifty-five other cases including five cases of extragastrointestinal cancer. While they have not had the opportunity to study early cases of gastrointestinal carcinoma, they are of opinion that the test will prove quite as definite in early cases as in late ones, and therefore will be of great value in early diagnosis.

Edwards[7] in 1919 reported the use of this test in twenty-nine cases of cancer, and found the curve reaches the neighborhood of two hundred milligrams at the end of forty-five minutes, and at the end of two hours is at approximately the same level, or may be considerably higher, than at the forty-five minute period. He concludes that failure to establish a "cancer" curve in the test is strong evidence against malignancy.

PERSONAL OBSERVATIONS

Our series of cases consists of 225 observations on 154 patients, fifty-three of whom had proved carcinoma. Our results correspond fairly closely with those of the workers just cited.

The test has been carried out as follows: Five cubic centimeters of blood is taken in the morning before the patient has had anything to eat; one hundred grams of anhydrous glucose is then given, dissolved in a cup of coffee; forty-five minutes from the time the glucose is taken five cubic centimeters of blood is drawn, and again at the end of two hours from the beginning. The amount of dextrose is then determined by the Folin-Wu[8] method. This consists essentially in the complete removal of the proteins from the diluted blood, reduction of a copper solution by the sugar in the filtrate, development of an intense blue color by a reaction between the reduced copper and a phosphomolybdate-phosphotungstate solution, and a comparison of this color with a standard dextrose solution similarly treated.

The results can be grouped in three fairly distinct classes as in the work of the previous observers.

Type 1 curve begins within or above the normal limits for a fasting individual, rises sharply to near two hundred milligrams at the end of forty-five minutes, and drops slightly, remains at the same level, or rises above the forty-five minute figure at the end of two hours. (Broken line, Fig. 1.) A certain number of atypical cases occur. These have the general characteristics of this curve except in that the rise is not so sharp or so high as in the more pronounced cases.

Type 2 curve, the normal curve, begins within normal limits, rises somewhat more gradually than does Type 1, and at the end of two hours has returned to near, or even below the original figure. (Solid line, Fig. 1).

Type 3 curve begins within normal limits, does not rise above the initial figure, and at the end of the test period is as low or lower than in the beginning. This is the type of curve we should expect to find in hypoactivity of the thyroid, suprarenals and pituitary glands. (Dotted line, Fig. 1.)

COMMENT

Table I shows the results of the test in 127 cases.

Factors affecting absorption from the intestinal tract have not been taken into consideration. Such factors may be responsible for the negative results in gastric carcinoma, as those cases giving Type 1 atypical curves were advanced cases with extensive involvement of the stomach.

Two cases of hyperthyroidism and two of hypothyroidism are tabulated. The results are exactly what we should expect. Four cases of neurasthenia are shown, all giving a curve exactly like that of hyperthyroidism. In neurasthenia there is hyperactivity of the thyroid. (Bandler.[9])

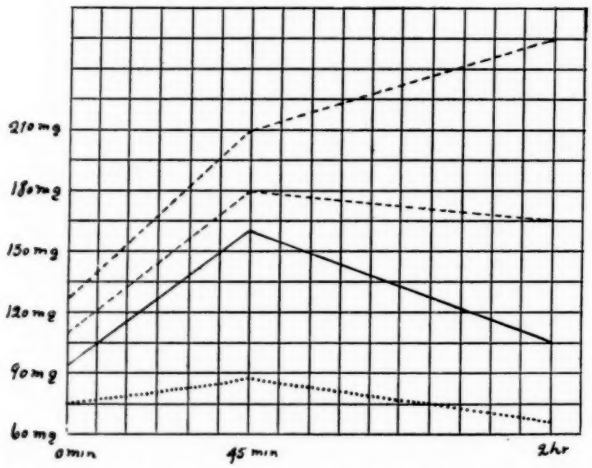

Fig. 1.—Broken line Type I, solid line Type II, dotted line Type III.

Diabetes mellitus invariably gives a Type 1 curve, and is remarkable for the height to which the blood sugar rises.

Of seven cases of sarcoma, three give a Type 1, four a Type 2 curve.

Of four cases of aortic aneurysm, all having a positive Wassermann, three give a Type 1 curve, in contrast to a number of other cases of syphilis, all of which give a Type 2 curve.

Of seven cases of jaundice from various causes, five, or 71 per cent show a Type 1 curve; therefore the test is of no value in suspected carcinoma complicated by jaundice.

Twenty-nine cases of tuberculosis are shown. Twenty, or 60 per cent show a Type 1 curve. Three out of six cases of very early incipient tuberculosis, or 50 per cent give this reaction. In one case, that of a young woman (worker in the laboratory) this was the first indication that she was abnormal. In running the test on a series of apparently normal individuals, this person was found to have a typical Type 1 curve. A few weeks later, when she

TABLE I

	TYPE 1 CURVE	TYPE 1 ATYPICAL CURVE	TYPE 2 CURVE	TYPE 3 CURVE
Carcinoma, Superficial	10	1		
Carcinoma, Gastric	3	3	2	
Carcinoma of Liver and Gall bladder	2	1		
Carcinoma of Colon	7			
Carcinoma of Rectum	4			
Carcinoma of Bladder	5			
Carcinoma of Prostate	5	1		
Carcinoma of Breast	2			
Carcinoma of Vagina	2			
Carcinoma of Cervix and Uterus	3		2	
Sarcoma, Various types	3		4	
Tuberculosis, Incipient pulmonary	3		3	
Tuberculosis, Advanced pulmonary	14	2	3	
Tuberculosis, Other than pulmonary	3	1		
Jaundice due to causes other than malignancy	5		2	
Osteomyelitis, Chronic	6	1		
Diabetes Mellitus	4			
Neurasthenia	4			
Hyperthyroid	2			
Hypothyroid				2
Gout				1
Hypertension, Essential				1
Obesity	1	1		
Cirrhosis of Liver	1		1	
Pernicious Anemia	1		1	
Aneurysm of Aorta	3		1	

called attention to her run down condition, tubercle bacilli were found in great numbers in her sputum.

Fifty-three cases of carcinoma are reported. These are all proved cases, either by tissue examination, postmortem or by unmistakable clinical signs. It will be noted that forty-three cases or 81 per cent give a typical Type 1 curve, (exactly the same type of reaction as is found in cases of hyperactivity of the thyroid, suprarenals, etc.), while six give an atypical Type 1 curve, and only four a Type 2. It is significant that in many cases there was a marked change in the type of the curve following removal of the tumor, in a few cases reaching normal within a month. In some cases, tissue examination revealed a very early malignancy, but the sugar curve was typical. This is significant.

Repeated tests have been run on numerous cases. The results fluctuate considerably, but invariably, the type is the same.

Carcinoma of the stomach gives the largest percentage of atypical Type 1 curves. In all these cases there is either extensive involvement of the stomach or obstruction of the pylorus with resulting retention of the sugar in the stomach; consequently the slow absorption and the low curve.

We have found only Type 2 reactions in some twenty other conditions not tabulated, including spastic colitis, ileocolitis, mastitis, lymphatic leucemia, hypertrophy of prostate, prostatic abscess, orchitis, urethral stricture with retention, acute tonsillitis, pancreatitis, salpingitis, malaria, dementia precox, acute articular rheumatism, arthritis deformans, obliterative arteritis, and syphilis.

A large number of determinations on normal individuals have given only Type 2 reactions.

Two explanations for the altered carbohydrate metabolism in carcinoma have been offered by Dr. Simon, quoted by Friedenwald and Grove[5]: 1, The increased demand of the growing tumor for carbohydrate; and 2, A hormone liberated by the carcinoma cells impairing the power of the tissues to oxidize sugar. We would suggest a third possibility, that a specific hormone is liberated by the carcinoma cells which acts to accelerate the process of glycogenolysis or retard the process of glycogenesis, or both, either directly, or by acting upon one or more of the endocrine organs or the vegetative nervous system.

CONCLUSIONS

Our series of cases is too small to form the basis of definite opinion. From a study of these results and of the literature we arrive at the following conclusions.

(1) Carbohydrate metabolism is under control of the internal secretions, and is sensitive to changes in endocrine function. Therefore blood sugar determinations may aid in the diagnosis of these conditions.

(2) Carbohydrate metabolism is disturbed by carcinomatous growth, apparently in the same way as in certain endocrine disturbances.

(3) This disturbance is probably due to a secretion of the tumor cells; consequently the effect may occur quite early.

(4) If this be true, the blood sugar tolerance test should be a valuable diagnostic test.

(5) There is no sugar tolerance curve definitely characteristic of carcinoma, but most cases of carcinoma give a certain type of curve which is found in comparatively few other conditions, including tuberculosis, diabetes mellitus and hyperthyroidism.

(6) The test is of no value in suspected carcinoma of the bile tract area complicated by jaundice.

(7) The test needs further confirmation.

I wish to acknowledge my indebtedness to the technicians in the Clinical Laboratories for their efficient work, and to the Staff of the University Hospital for the privilege of studying their cases.

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AN IMPROVED METHOD OF CAGING AND FEEDING MICE*

By Helen S. Mitchell, New Haven, Conn.

ALTHOUGH the caging and feeding of mice is a common laboratory procedure, a particularly convenient form of individual mouse cage which facilitates handling and makes possible an accurate determination of food intake may be of interest to other workers in this line.

The wire cage without the special feeding device has previously been used by Wheeler[1] in this laboratory. Views of the separate parts and the assembled cage are shown in the accompanying photographs. In the top a trap door large enough to admit a person's hand makes it possible to introduce material into the cage without a chance for the animals to escape. A detached shallow pan in which is inserted a sheet of absorbent paper covered by a corresponding square of fine wire gauze, forms the floor of the cage and can be easily cleaned and sterilized. The paper serves to retain the urine voided and the wire gauze prevents the animals from tearing or gnawing the paper.

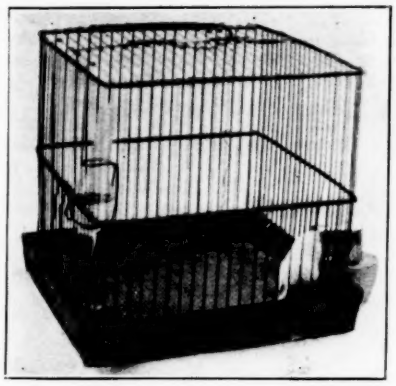

Fig. 1.—Showing the experimental cage and feeding tube in use.

Most investigators have experienced difficulties in feeding mice because these animals scatter their food and thus prevent an accurate determination of food intake. The use of a paste food which has been found advantageous for rats was unsatisfactory in the case of mice which invariably pawed the whole cup-full out onto the floor of the cage. To avert such spilling of food a very simple device was suggested by the more elaborate outfit for rat-feeding described by Hopkins and Ackroyd[2] and Macallum.[3] This device was easily attached to our wire cages and proved most satisfactory in preventing scattering and in keeping the food free from urine and feces.

*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.
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An elbow of ¾ inch tin pipe was cut to such a length that the horizontal section extended out over the edge of the pan and the vertical part reached down into a porcelain crucible used as food cup. A vertical cage wire was cut to permit the insertion of the feeding tube. The bent ends of the wire and the flanges on the tube prevented the dislodgment of the latter. The paste food tended to hold both the cup and feeding tube firmly in place and thus hindered

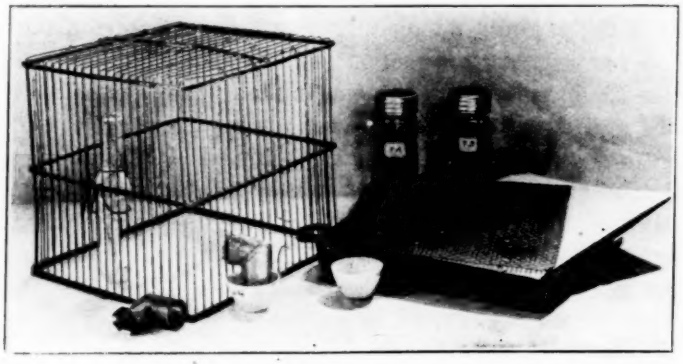

Fig. 2.—Showing the individual parts of the mouse cages and the feeding devices used for experimental animals.

the mouse from pushing the cup away and escaping. To make the device most successful it was found advisable to make the food in the form of a firm paste and to keep the cups well filled.

The expense of elaborating these cages was defrayed by a grant from the Russell H. Chittenden Research Fund for Physiological Chemistry. They were manufactured by the Andrew B. Hendryx Co., New Haven, Conn.

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EDITORIALS

Basal Metabolism

THE determination of the basal metabolic rate in routine clinical work is now possible, even for the private patient at home. New terms have come into use which the general practitioner is expected to know and use. An attempt will be made to explain the meaning of these terms and to describe briefly the principles underlying the determination of the basal metabolic rate.

The body is made up of cells, each of which must carry on and take its place in the performance of body functions. There are certain processes, chemical and physicochemical, going on within the cell all the time and upon these processes the life of the cell depends.

It is necessary for a city to have an influx of food and fuel and also to have an adequate waste disposal system; so the individual, a city of cells, must have a supply of food and fuel carried to the cells by the blood and a waste disposal system, again the blood stream, to carry the waste products CO_2, urea, etc., to the disposal stations, the kidneys, lungs and skin.

The cells take up the food brought to them by the blood, synthesize the

food elements into their own structure. The cells themselves are then oxidized, giving off carbon dioxide and water. To these chemical and physicochemical processes going on within the cells has been applied the term "metabolism." This term, in the original, means "change" and in the dictionary it is defined as that process by which living cells incorporate the matters obtained from food into their own structure. The rather limited normal metabolism of the body cells is dependent on an adequate supply of oxygen and the adequate removal of the carbon dioxide.

The amount of metabolism is affected by three groups of processes. These are, 1st. The vital processes such as respiration and circulation. 2nd. Digestion. 3rd. Muscular exertion. The amount of the metabolism is the least when the latter two factors are eliminated as far as possible allowing the first group to reach a minimum. This low line of metabolism is spoken of as "basal" metabolism. It is obtained by having the patient abstain from eating or drinking anything other than water for twelve hours or more and by requiring him to lie quietly for at least thirty minutes prior to performing the test for the determination of the basal metabolic rate. This rate is usually expressed in the number of heat units or calories produced by the individual per hour per square meter of body surface. The latter is determined from the nude weight and height of the patient. This rate may then be compared with the rates which have been determined for a large series of healthy individuals of both sexes and of varying ages, and the result of the comparison is expressed as percentage above or below the normal. Eighty-six per cent of all normal persons have a basal metabolic rate which is within 10 per cent of the curve for normals.

There are two principal methods of determining the metabolic rate; direct and indirect calorimetry, the one depending on the direct measurement of the heat loss of the individual, the other being computed from the oxygen consumption, the carbon dioxide liberated, and the nitrogen output in the urine.

The two methods are combined in the large respiration calorimeters of the Sage Foundation, the Mayo Clinic, and other large institutions. Briefly this calorimeter consists of a closed room surrounded by water, the weight and temperature of which is known throughout the experiment and from this the amount of the heat loss from the patient is measured directly. While in the calorimeter the patient is supplied with air having a definite oxygen content. By means of a powerful blower or fan the air is kept in circulation and is forced from the room through sulphuric acid which removes the moisture, then through soda-lime which removes the carbon dioxide, and then through another bottle of sulphuric acid to remove the moisture absorbed from the soda-lime. By weighing accurately these different substances before and after the experiment one can determine the exact amount of the oxygen used, the amount of moisture and the amount of carbon dioxide liberated by the individual. The ratio of the volume of the CO_2 liberated to the volume of the O absorbed is known as the respiratory quotient and its value depends upon the type of food which is being consumed or metabolized by the body cells. Thus

for carbohydrates the volume of the CO_2 liberated by their oxidation is the same as the volume of O consumed and the respiratory quotient is 1.

1. $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$.

2. 6 volumes of CO_2 divided by 6 volumes of O equals 1. While for fats there is not enough oxygen in the molecule to form water hence some of the oxygen used is for that purpose, resulting in the formation of fewer volumes of carbon dioxide than of oxygen used. Taking tristearine as an example:—

1. $C_{57}H_{110}O_6 - 6H_2O = C_{57}H_{98}$.

To form carbon dioxide and water from the $C_{57}H_{98}$ will require 81.5 volumes of oxygen.

2. $C_{57}H_{98} + 81.5$ vol. $O_2 = 57CO_2 + 49H_2O$.

3. $\frac{57 \text{ vol. } CO_2}{81.5 \text{ vol. } O_2}$ 0.7 the r.q. (respiratory quotient) for fat.

For proteins, on the average, the respiratory quotient is about 0.81. Knowing the respiratory quotient one can determine which of the food elements is being metabolized. Each gram of protein or carbohydrate when oxidized liberates 4 calories of heat, while fat liberates about 9 calories per gram burned. The amount of these food elements which may be oxidized by a liter of oxygen has been determined and from this the caloric value may be found (if the r.q. is known). This figure, the caloric value of 1 liter, multiplied by the number of liters used and divided by the surface area of the patient will give the number of calories per hour per square meter of body surface, and is known as the basal metabolic rate. This latter method of indirectly determining the rate from the oxygen and the carbon dioxide requires relatively less complicated apparatus than the respiration calorimeter.

The two methods above described, direct and indirect calorimetry, were found to agree very closely, and this has led to the development of smaller machines for the indirect determination of the basal metabolic rate. These smaller machines are of two types, the one determining both the oxygen intake and the carbon dioxide output and from these findings determining the r.q. and the resulting caloric value of the oxygen used. The other type of machine determines only the oxygen consumption and, using a constant r.q. of 0.82, one may determine the caloric value of the oxygen used. The determinations of the basal metabolic rates on the same individual at the same time with these various types of metabolimeters agree within 5 per cent, and are therefore sufficiently accurate for clinical purposes. This is especially true if the determinations are made by one who is entirely cognizant of the difficulties and of the large "personal equation" which necessarily enters into such determinations.

For persons in health, the normal basal metabolic rates figured in calories per hour per square meter of body surface have been figured out by Aub and Dubois and are as follows:—

AGE	MALE	FEMALE	AGE	MALE	FEMALE
6-8	58	58	20-30	39.5	37.5
8-10	54	54	30-40	38.5	36.5
10-12	52	50	40-50	38.5	36.0
12-14	50	46	50-60	37.5	35
14-16	46	43	60-70	36.5	34
16-18	43	40	70-80	35.5	33
18-20	41	38			

Variations of more than ten per cent from the above findings are considered pathologic. Variations of less than ten per cent are considered physiologic.

With relation to basal metabolic rate there are three classes of disease, i.e.—1st. Those diseases having a normal basal metabolic rate. 2nd. Those diseases having an increased basal metabolic rate. 3rd. Those diseases having a decreased basal metabolic rate. The first class includes those afebrile diseases not of endocrine origin, as, for example, neurasthenia, the nephritides, and cardiac cases which are still in a state of compensation. The second class includes all febrile diseases, those diseases of the endocrine system that are characterized by an increased secretion of the gland at fault, the leukemias, pernicious anemia, paralysis agitans, dyspneic cardiorenal cases, and those cases of diabetes which have an increased protein metabolism. The third class referred to is composed, for the most part, of cases in which there is a decrease in the quantity or quality of the secretion of one or more of the endocrine glands. Cases of cretinism and of myxedema come in this class. Cases of undernutrition will also often show a slight lowering of the basal metabolic rate.

The amount of the increase of the rate in the diseases of the second group may be used as an index to the severity of the disturbance. After ruling out fever as a cause of the increased rate about 90 per cent of all the marked increases found are due to hypersecretion by the thyroid gland. These cases may be divided into two groups, the adenomas of the thyroid with hyperthyroidism and the hyperplastic thyroids with hyperthyroidism. Those of the latter group come on at an earlier age, have a more acute onset, and as a rule have a higher basal metabolic rate. From the standpoint of the basal metabolic rate hyperthyroidism may be grouped into the very severe with an increase of 75 per cent or more, moderately severe having an increase of between 50 per cent and 75 per cent, and mild, including cases having an increased rate up to 50 per cent. Hypersecretion of the posterior lobe of the hypophysis will also produce a marked increase of the basal metabolism while the bilobar disturbance of Fröhlichs disease usually does not cause a change of the basal rate from the normal.

In leukemia the basal metabolic rate may be increased to 40 per cent or 50 per cent with but little clinical evidence of the disturbance of metabolism. It is worth noting that these cases do not show an improvement of the rate

after therapy (such as x-ray of the spleen and the long bones) which causes a marked decrease in the number of pathologic leucocytes. In pernicious anemia the basal metabolic rate may be normal or it may be increased up to 30 per cent. The explanation which has been offered for the increased basal metabolism in leukemia and in pernicious anemia is the large and continuous increase of embryonic white and red cells.

Paralysis agitans alone may give an increased basal metabolic rate up to 50 per cent. In these cases the rise in oxygen consumption is most probably due to the constant muscular tremor with a consequent oxidation of muscle substance. When a case has both paralysis agitans and hyperthyroid disturbance the basal metabolic rate may be very high. One such case coming under our observation showed an increase of 121 per cent. This case was reported as markedly improved following x-ray treatment of the thyroid gland.

Cardiorenal cases which have reached the stage of decompensation may, with the labored breathing, cause an increase of the basal metabolic rate up to 50 per cent.

Diabetic cases may show a normal, an increased, or even in some cases a decreased basal metabolism. The increase usually accompanies an increase in the metabolism of the body proteins.

Those cases showing decreased metabolic rates usually have a definite clinical picture which points toward the diagnosis. The thyroid and the pituitary glands are the glands most often at fault. Snell and others have reported a series of 13 cases of cretinism and myxedema. These cases showed rates varying from –7 per cent to –26 per cent. Sandiford has also reported several cases, one of which had a basal metabolic rate of 40 per cent below the normal. Thyroid therapy brings the rate up to normal in many of these cases but as the rate increases many of them show all the signs of hyperthyroidism even before the rate reaches normal. Undernutrition usually does not cause a marked decrease of the basal metabolic rate.

One uses a thermometer to determine the rise and decline of a fever temperature. One should use the metabolimeter to determine the extent of the change in the basal metabolic rate and to note the effects of treatment whether that treatment be medical, x-ray, or surgical. Used in this manner it is one of the most valuable additions for the study of diseases of endocrine origin.

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—*H. L. C. (per P. G. W.)*

Intimate Exposure and Contagion in Tuberculosis

INORDINATELY difficult to estimate is the relative importance of heredity and family exposure in propagating tuberculosis.

It is pretty clear, in spite of an occasional dissenting voice, that the children of the tuberculous too often develop the disease. Our records at Cragmore Sanatorium, in Colorado Springs, show a known and acknowledged positive family history in over 50 per cent of the cases.[1] We very often learn of the disease pursuing a family through three or more generations, and this among intelligent and careful people living in the best circumstances. Not infrequently investigation discloses that a parent or grandparent is an unsuspected chronic disseminator of tubercle bacilli.

Adams,[2] in an endeavor to show that family tuberculosis is not common, reports the family history negative in 60 per cent of cases—that is, positive in 40 per cent. Solis-Cohen[3] speaks of securing positive family histories in a little more than 40 per cent of a group of patients.

If an accurate general census could be made of all persons, regardless of the state of their health, approximately one in five, or 20 per cent, should give a history of tuberculosis in at least one parent, since about one person in ten dies of the disease. The additional percentage of those who are known to have tuberculosis, but recover and die from other causes, is small. The estimate may therefore fairly be based on the mortality percentage, doubled for the two parents. The figure would be increased by including other relatives besides parents, but is in reality reduced by lack of knowledge and lack of candor in our patients, so that it is likely that the latter source of error more than balances the former. As a rough estimate, 20 per cent may be taken as the approximate expectation of a family history of tuberculosis in the population at large.

We have then among the tuberculous a percentage of positive family histories more than double the normal expectation. The family grouping of the disease is striking. Illustrations of this are abundant, and some excellent ones are given by McBrayer[4] in a report of tuberculosis in a village in North Carolina.

But, granted the occurrence of family tuberculosis, how are we to interpret it? Do the children of those whose resistance to tuberculosis has been inadequate inherit a faulty resistance, or is it merely a matter of excessively large and frequent infection, or do both factors operate? Adams, and King, whom he quotes, feel that far from having a lower resistance, persons with positive family histories have a higher resistance, a greater tendency to recovery and chronicity, than others who contract tuberculosis have. There may be truth in this observation, if only adult victims of phthisis are considered, for the adult tuberculous invalid with a positive family history is often a survivor, many of whose less resistant brothers and sisters have already died of acute tuberculosis in childhood.

Against the hypothesis of special susceptibility inherited from tuberculous parents stand the reports of the Oeuvre Grancher.[5] This institution, which removes the children of the tuberculous from their parents and cares

for them in healthful surroundings, reports a very low incidence of tuberculosis among its charges. An identical observation has been made by Calmette[6] in cattle. The low tuberculosis mortality among the native population of Colorado Springs, many of whom are the children of tuberculous parents, has been emphasized by Gardiner,[7] and is evidence to the same effect. For equipoise, we ought to have reports of children from healthy families who have been brought up in close contact with the tuberculous. This often happens, and some cases of resulting disease have been reported,[6] but no large body of data has been collected.

We agree with the conventional creed of contemporary phthisiology in believing early exposure to be of the gravest importance, though we believe also that there are very great and significant racial and individual variations in the power to cope with the disease, and that these appear very early in life—a subject which calls for more ample discussion. Raymond Pearl,[8] one of the foremost students of this problem, says, "Familial contact with active open cases is beyond question a factor in determining the incidence rate of clinically active tuberculosis. It appears equally obvious, however, that it certainly does not account for the whole, and probably accounts only for a small part, of the increase in the incidence of the disease which we find to occur as the amount of tuberculosis in the immediate direct ancestry increases."

Allowing that intimate exposure in infancy and childhood is likely to be disastrous, there is the still more debated question as to the possible danger of intimate exposure in adult life.

Marriage is of course the most intimate of adult relationships, and the lack of unanimity as to the occurrence of marital contagion in tuberculosis seems to be evidence that this is, on the whole, not so very frequent. Here again there are many complicating and confusing factors. Special emphasis has been laid on the tendency of similar persons to select each other—the "assortive mating" of Karl Pearson. This, however, is flatly denied by Ward,[9] whose numerous communications all present data in favor of frequent adult contagion. Ward thinks that people with tuberculosis are most likely to marry individuals of entirely different type.

Lawrason Brown[10] has indicated one of the reasons for our confusion on this subject, namely the fact that many of the reports dealing with the matter are not based on the whole life history of the exposed mates, and their final mortality, but on the incidence of the disease in a short period of observation. An investigation of his own, in which this error was eliminated, covered forty thousand couples and led to the conclusion that there was an excessive incidence of tuberculosis among the marital partners of the tuberculous, but that possibly two-thirds of this was due to assortive mating. Brown himself, however, believes that adult contagion is important.

Barnes[11] has recently reported that "the histories of 229 consecutive widowed patients admitted to the Rhode Island State Sanatorium 1905-1921, show that 93, or 40 per cent, lost their consorts by death from tuberculosis, a

tuberculosis mortality over three times that of the married people of the community."

Crouch, at the Modern Woodmen Sanatorium in Colorado Springs, found that 58 per cent of 233 widower patients had lost their wives through tuberculosis. Minnig,[12] who quotes these figures, gives 50 per cent as his own observation.

Against these positive reports we must weigh the great body of statistics from hospitals and sanatoria, showing a lack of any excess tuberculosis morbidity and mortality among those who care for the tuberculous. Here, however, the contact is not very intimate—at least it does not combine intimacy with perpetuity as marriage does.

Perhaps the various statistical researches which are now under way will illuminate this dark and disputatious corner of our ignorance. At present one feels that expert opinion, which changes so often, is moving in the direction of more emphasis on the possible danger of very intimate and prolonged exposure to the tuberculous invalid, even in adult life, and this in spite of much erudite argument to the contrary.

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G. B. W.—(C. T. R.)

Sputum Examination

SPECIMENS of sputum are examined less carefully, as a rule, than any other material from the body, possibly excepting the feces, and yet a considerable amount of information can be obtained from the study of it, provided the specimens are properly obtained. Too frequently specimens are mixtures of buccal, pharyngeal and nasopharyngeal secretions with some admixture of bronchial material. Examination of such mixed specimens is, if not valueless from a pulmonary standpoint, at least misleading.

On the other hand studies of sputum raised by a true pulmonary coughing act may give information which, taken together with x-ray studies of chest plates, is exceedingly valuable for diagnosis, prognosis, and treatment.

There is no need to recount the value of sputum examinations in tuberculosis, except to note that modern methods of digestion of the material with alkali followed by centrifugalization (Raphael and Eldridge) gives more accurate results than the older method of merely smearing the material on slides.

In asthma too, very useful information may be obtained in making a differential diagnosis.

Asthma may be of several origins. It may be a disease *sui generis*. It may be a symptom of renal, or cardiac, or pulmonary disease. The characteristic laboratory finding in true bronchial asthma is eosinophilia in the sputum, and this according to Bezancon and Dr. Jong has almost as much value in the diagnosis of asthma as the finding of tubercle bacilli in the diagnosis of tuberculosis. According to these writers Charcot-Leyden crystals, and Curschmann's spirals are of little significance. Also they say the sputum in true asthma is free from albumin which appears only in complicated cases. It is especially in men over 50 who present attacks of nocturnal dyspnea of an asthmatic character that sputum examinations have a special value in determining whether the disease is true asthma, or whether it is a cardiovascular affair.

It is not, however, only in tuberculosis and asthma that microscopic study of the sputum is of real diagnostic value. In bronchial spirochetosis, for instance, a disease which in many respects resembles influenzal bronchitis, certain types of pneumonia and tuberculosis, the diagnosis can only be established by careful study of the sputum. Mason has recently reported a case of this infection in which the spirochetes were found in a pleural empyema. Rapid improvement followed intravenous salvarsan. Pulmonary streptothricosis is another disease which, though it may resemble clinically other types of lung infection, for instance tuberculosis, is due to the presence of filamentous organisms which show true branching. Such cases are sometimes referred to as pseudotuberculosis. Bridge in California has reported fifteen cases of pulmonary streptothricosis. In some of his cases the streptothrix was associated with the tubercle bacillus. In one of Zenoni and Macchi's cases there was a pure streptothrix infection. In pure cases there was no tendency for the disease to begin in the apex of the lung as in tuberculosis; the fever was rarely high, and emaciation was not rapid. Hamman says of these cases that the clinical symptoms are those of tuberculosis except for the localization in the lower lobes. In such cases both sputum examinations and radiograms have a place.

Occasionally certain species of molds attack the lungs, most frequently that known as aspergillus fumigatus. Usually it is a secondary invader in tuberculous cases, but as Hamman says it may affect the lungs primarily causing ulcerative and suppurating lesions. Aspergillus fumigatus is widely distributed in the outer world and may be found on vegetables, grain bread, and other food stuffs. It is presumed that infection follows inhalation of the spores. There is nothing characteristic in the clinical picture of aspergillosis, and a diagnosis can only be made by microscopic examination of the sputum. In primarily tuberculous cases the organism adds nothing important to the clinical symptoms. When the infection is primary the symptoms are those of chronic tuberculosis. A bronchial form which runs the clinical course of a chronic bronchitis has been described.

Pulmonary actinomyces is comparatively rare, but the microscopic find-

ings are characteristic. In the early stages the diagnosis can be made only by discovering the organism in the sputum. In the later stages it is difficult to distinguish it from tuberculosis, carcinoma, bronchiectasis, abscess, etc., without the characteristic microscopic findings.

Since the last great epidemic of influenza many cases of chronic bronchial and lung diseases have appeared that are often difficult to diagnosticate without careful bacteriologic examination of the sputum. Many such cases resemble tuberculous disease, but because of the consistent absence of tubercle bacilli they have been called "localized bronchitis," "subacute and chronic nontuberculous lung infection," "a lobular form of bronchopneumonia of long duration," etc. Field in discussing such cases says that the onset may occur at any age, but is commonest in children. The syndrome usually follows acute disease, which particularly affects the upper respiratory tract, such as measles, whooping-cough, and influenza. In some cases it comes on insidiously. Both the onset and exacerbations occur most frequently during the colder months, probably owing to crowding in ill-ventilated rooms. Chronic sinusitis is probably present in all the cases. B. influenza is the most frequent predominating organism, but any of the common invaders, streptococci, staphylococci, pneumococci, and micrococcus catarrhalis, may appear. The process tends to pulmonary fibrosis, or, later, to bronchiectasis and interstitial pneumonia. In these cases the x-ray is of assistance only in locating the lesions, which are usually not typical as in tuberculosis.

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—*P. G. W.*